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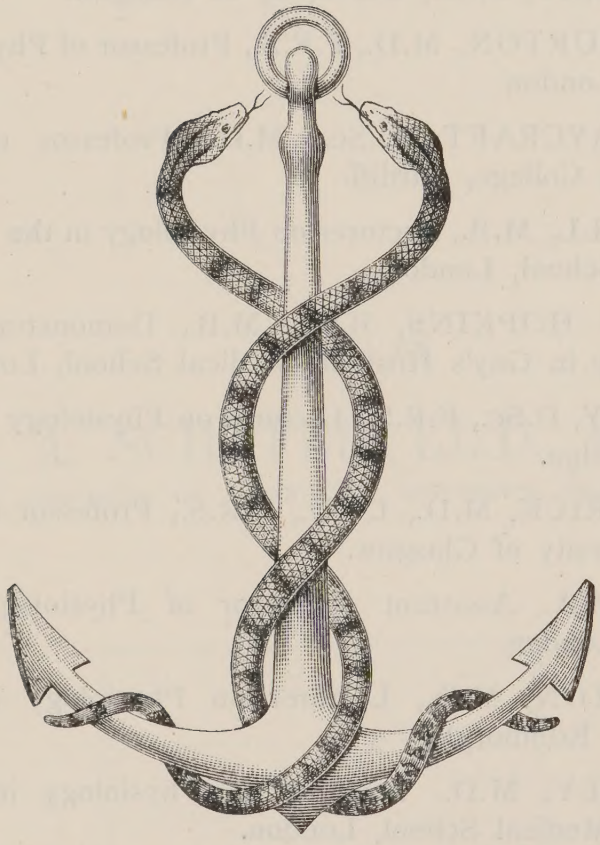

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TEXT-BOOK

OF

PHYSIOLOGY

EDITED BY

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VOLUME FIRST.

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PREFACE.

THE want of a text-book in the English language to which students could turn for information beyond that contained in the ordinary manuals has long been felt by teachers of physiology in this country. The most extensive of the existing text-books do not aim at giving the full and precise information nor the references to original authorities which are required by the advanced student. It has hitherto been necessary for those who seek such information to consult original articles—an operation which frequently involves a familiar acquaintance with foreign languages and an expenditure of time rarely at the disposal of the student. The present work is not intended altogether to supersede this consultation of original papers, but will, it is hoped, reduce the need of it to more reasonable limits, and will, moreover, by the references to literature which throughout form an important feature of each article, facilitate such study where it is still necessary.

A book of this character, from the enormous amount of literary labour which is involved in its production, and from the progressive character of the science with which it deals, could hardly be undertaken by one person. The editor has been fortunate enough to secure the co-operation of many of the leading physiologists in this country, each of whom deals with some branch of the subject to which he has given special attention. Accordingly the reader will find in each article, in addition to information as to the present state of knowledge as complete as it has been possible to make it, many original observations upon the matter to which it relates.

The subjects of generation and reproduction have been omitted in this text-book, because, although strictly speaking appertaining to physiology, they are studied almost entirely by morphological methods, and are more conveniently treated in connection with morphology. It has therefore been decided that it would be better not to swell the bulk of these volumes, which have already grown beyond the limits originally intended, by the introduction of subjects such as these, which possess an enormous recent literature, and are exhaustively dealt with in special works accessible to every student. The same remark will apply to the general physiology of the cell, a branch of biology which has

of late attained so great an extent and importance as to necessitate text-books devoted to itself alone, and which it is usual to study rather as an introduction to, than as a part of, animal physiology.

Of the two volumes of which it is intended this book shall consist, the articles in the first volume deal mainly with the chemical constitution and the chemical processes of the animal body, and with those physical and chemical phenomena which are connected with the production and elaboration of the secretions and other fluids of the body. The articles in the second volume include the mechanics of the circulation and respiration, and of special muscular movements; the general physiology of muscle and nerve; the special senses; and the functions of the central nervous system.

It is nearly twenty years since the publication in six volumes of the important "Handbuch der Physiologie," under the editorship of Professor L. Hermann. The articles in that book, as in this, were undertaken by physiologists who were specially conversant with the particular branches of the science with which they severally dealt; and since most of the articles in it are prefaced by short historical introductions, and interspersed with abundant references to the literature of the subject, the whole work constitutes a storehouse of information, which has proved of great value to teachers and investigators. But the size of the work, and the fact that it is written in the German language, have limited its utility to students in this country; moreover, in the course of the twenty years that have elapsed since its appearance, rapid progress has been made in every branch of physiology, so that several of the articles in it have been long out of date. Nevertheless its publication served both to lay a firm foundation for the exposition of the science in its modern aspect, and also to clear the ground for all future publications of a similar character. It has thus been a marked advantage, in preparing many of the articles for the present book, to have had the work of Hermann and his coadjutors to refer to; and although due acknowledgment is made both of this and of other sources of information in the articles themselves, it has seemed right specially to mention the "Handbuch" in this preface.

University College, London,
February 1898.

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TEXT-BOOK OF PHYSIOLOGY.

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THE CHEMICAL CONSTITUENTS OF THE BODY AND FOOD.

By W. D. Halliburton.

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The chemical constituents of the body are very numerous, and the majority of them are compounds of complicated structure. In the following article I propose to treat of these compounds, first in classes, and then individually, and in a subsequent chapter to discuss the various tissues and organs in their chemico-physiological aspects.

In order to classify the chemical constituents of the body, one might proceed upon a purely chemical basis, beginning with the simplest and ending with the most complex compounds; or a purely physiological basis might be adopted, in which the compounds would be described in the order of their importance in the vital processes of the organism. But a compromise between these two exclusive methods is found to be that which is of most practical usefulness.

We may, in the first place, divide the compounds found in the body into those of *inorganic*, or mineral nature; and those which are termed *organic*, or carbon compounds.

The inorganic compounds present are water; various acids, such as the hydrochloric acid of the gastric juice; and numerous salts, such as calcium phosphate in bone, and sodium chloride in blood, urine, etc.

The organic compounds are more numerous, and these we may conveniently group as follows:—

Nitrogenous .	Proteids, *e.g.* albumin, myosin.
	Albuminoids, *e.g.* gelatin, keratin.
	Simpler nitrogenous substances, *e.g.* lecithin, creatine.
Non-nitrogenous	Fats.
	Carbohydrates, *e.g.* sugar, starch.
	Simpler organic substances, *e.g.* alcohols, lactic acid.

The most useful classification of the more complex organic compounds is the time-honoured one, into proteids, carbohydrates, and fats. Taking this as our starting-point, we shall find that the other substances present may be described either in subsidiary classes to these, or as decomposition products of the more complex substances.

The elements found in these compounds are carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, chlorine, iodine, fluorine, silicon, sodium, potassium, calcium, magnesium, lithium, iron, and occasionally manganese, copper, and lead.

It will be on the whole most convenient to study the organic compounds first, in the following order:—

1. Carbohydrates;
2. Fats, with which we shall study the lecithins and cholesterins;
3. Proteids and albuminoids.

In following out this plan we shall discuss some of the chemical constituents of the food as well as those of the body.

The Carbohydrates.

The carbohydrates are found chiefly in vegetable tissues, and many of them form important foods. Some, however, are found in or formed by the animal organism, such as glycogen or animal starch, dextrose, and lactose or milk-sugar. The carbohydrates may be conveniently but loosely defined as compounds of carbon, hydrogen, and oxygen, the two last-named elements being in the proportion in which they occur in water. But this definition, if pushed, would include several substances like inosite, acetic acid, and lactic acid, which are not carbohydrates.

The work of Fischer,[1] Tollens,[2] and many other chemists has, moreover, shown that carbohydrates are not, as their name would imply, simply compounds of carbon with water, but their constitutional formula has been in many cases thoroughly worked out, and their composition shown to be much more complex. This work has culminated in the synthetical production of many of the sugars.

From the chemical standpoint, the sugars (which are the simplest of the carbohydrates) may be divided into two classes—

1. Those which, when digested with dilute acids, do not yield any other sugar or sugars; this class includes the glucoses; and

2. Those which, when so treated, do yield some other sugar or sugars; this class includes the members of the cane-sugar group.

Further, the sugars are designated according to the number of carbon atoms they contain; thus we have trioses (*e.g.* glycerose), tetroses (*e.g.* erythrose), pentoses (*e.g.* arabinose, xylose, rhamnose), hexoses (*e.g.* glucose, mannose), heptoses, octoses, and nonoses, according as they contain, three, four, five, six, seven, eight, and nine atoms of carbon respectively in their molecules.

The great majority of these sugars possess, however, but little

[1] See especially E. Fischer, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxiii. S. 2114.
[2] Tollens, "Kurzes Handbuch der Kohlenhydrate," Breslau.

physiological interest, and their chemical relationships and reactions will be found described in works on chemistry.[1]

Those which are of physiological importance are the hexoses and their derivatives. Nearly all the carbohydrates with which we have to deal in the animal body contain either six carbon atoms, or some multiple of six. The same is true of those which are used as food. The remainder are either synthetical products of the chemical laboratory, or more or less rare products of the vegetable world.

But to this rule there is one exception; the pentoses do possess some physiological importance. When Hammarsten[2] was investigating the nucleo-proteid material he separated from the pancreas, he found that by boiling it with dilute mineral acid he obtained a reducing substance. This formation of a reducing sugar-like substance from nuclein is not unique, as Kossel[3] and his pupils have obtained a similar product from yeast-nuclein. The sugar, however, does not ferment with yeast, but, like the pentoses, gives a red coloration with phloroglucinol and hydrochloric acid, and by distillation with hydrochloric acid yields furfuraldehyde. An osazone is obtainable from it in the form of fine rosettes of crystals, melting at 158° to 160° C., and these appear to be identical with those prepared from pentoses by E. Salkowski and M. Jastrowitz.[4]

The physiological action of pentoses was investigated by W. Ebstein.[5] When xylose or arabinose, dissolved in water or coffee, are taken with the food, they rapidly appear in the urine; they are not assimilated. The use of fruits, such as pears, that contain *pentosanes*, the mother substances of pentoses, may lead to the appearance of the latter substances in the urine. It is of course important not to confound such a temporary condition with diabetes.

Max Cremer[6] has investigated the physiological action of some of the rare sugars, especially their influence on the formation of glycogen. He found that in rabbits mannose increases the hepatic glycogen, and that, though the pentoses readily pass into the urine, a small quantity is assimilated as glycogen. Lindeman and May[7] have confirmed Cremer's results.

Salkowski[8] has investigated a large number of diabetic urines, but was unable to find pentose in any of them. Nevertheless, he found pentose in various other morbid conditions in the urine, in which their presence could not be attributed to diet. He suggests that in these cases they originate in the body from such nucleo-proteids as Hammarsten found in the pancreas, the processes of oxidation being lessened so that they were not broken up into simpler materials.

We can now proceed to the study of the carbohydrates concerning which we have more accurate physiological knowledge; and these may be classified into the following three groups:—

[1] See article "Sugars," Watts's "Dictionary of Chemistry," London, 1894, vol. iv.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xix. S. 19.

[3] Kossel and Neumann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxvii. S. 2215.

[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1892, Nos. 19 and 32. Blumenthal (*Berl. klin. Wchnschr.*, 1897, Bd. xxxiv. S. 245) has obtained pentoses from numerous other nucleo-proteids.

[5] *Virchow's Archiv*, Bde. cxxix. S. 401; cxxxii. S. 368; cxxxiv. S. 361.

[6] *Ztschr. f. Biol.*, München, Bd. xxix. S. 484.

[7] *Chem. Centr.-Bl.*, Leipzig, 1896, Bd. i. S. 932.

[8] *Berl. klin. Wchnschr.*, Bd. xxxii. S. 364. See also Külz and Vogel (*Ztschr. f. Biol.*, München, 1895, Bd. xxxii. S. 185). These observers found pentoses in only four out of sixty-four cases of human diabetes. But they are generally found in the severe forms of diabetes produced in dogs by the extirpation of the pancreas or by administration of phloridzin.

1. *Monosaccharides* ($C_6H_{12}O_6$).—The most important members of this group are :

Dextrose.	Galactose.
Levulose.	Mannose.

2. *Disaccharides* ($C_{12}H_{22}O_{11}$).—The most important members of this group are :

Cane Sugar.	Maltose.
Lactose.	Isomaltose.

3. *Polysaccharides* ($C_6H_{10}O_5)_n$.—The most important members of this group are :

Starch.	Cellulose.
Glycogen.	Tunicin.
Dextrins.	Gums.
Inulin.	

The monosaccharides.—When an alcohol is oxidised, the first stage in oxidation is the formation of an aldehyde, or a ketone; if oxidation of the aldehyde is continued, an acid is formed.

When more complicated alcohols are oxidised, similar products result. The monosaccharides are the first oxidation products of the hexatomic alcohols ($CH_2.OH—(CH.OH)_4—CH_2OH$).

Of the hexatomic alcohols, three are known, namely sorbite, mannite, and dulcite.

Dextrose is the aldehyde of sorbite.[1]
Mannose „ „ mannite.
Galactose „ „ dulcite.
Levulose „ ketone of mannite.

Sugars of the monosaccharide group may thus be either aldehydes, when they are called *aldoses*; or ketones, when they are called *ketoses.*

Dextrose, mannose, and galactose are aldoses, and have the structure represented by the following formula :—

$$CH_2.OH—(CHOH)_4—CHO$$

They differ from one another in their stereochemical formulæ.

Levulose is a ketose, and has the structure represented by—

$$CH_2.OH—(CH.OH.)_3—CO—CH._2—OH$$

The difference between the aldoses and ketoses is shown by oxidation, levulose, like all ketoses, yielding acids which are poorer in carbon.

If chlorine or bromine water is used as the oxidising agent, the *aldoses* (dextrose, mannose, and galactose) give isomeric monobasic acids of the formula—

$$CH_2.OH—(CH.OH)_4—COOH;$$

and then, by further oxidation by means of nitric acid, yield dibasic acids of the formula—

$$COOH—(CH.OH)_4—COOH$$

Both sets of acids are stereo-isomerides.

	Monobasic acid.	Dibasic acid.
From Dextrose . .	Gluconic acid	Saccharic acid.
„ Mannose . .	Mannonic acid	Manosaccharic acid.
„ Galactose . .	Galactonic acid	Mucic acid.

[1] Meunier, *Compt. rend. Acad. d. sc.*, Paris, tome cxi. p. 49; Vincent and Delachanal, *ibid.*, p. 51.

Glycuronic acid.—If saccharic acid is heated five or six hours in the water bath, it is changed into saccharo-lactonic acid, $C_6H_8O_7$. If this is reduced by means of sodium amalgam, one obtains *glycuronic acid*;[1] this substance is of considerable interest because it is sometimes found in the body, and when it passes into the urine is apt to be mistaken for sugar, many of the tests for which it gives.

Its composition is—

$$COOH—(CH.OH)_4—CHO = C_6H_{10}O_7$$

It is soluble in water and alcohol, is dextro-rotatory, reduces alkaline solutions of metallic salts, and yields saccharic acid on oxidation with bromine. It does not undergo the alcoholic fermentation. Though related in its composition so nearly to the carbohydrates, it yields with urea decomposition products which are aromatic, such as orthonitro-benzyl alcohol (Jaffe).[2] It occurs in the urine in the form of the potassium salt ($C_6H_9O_7K$) after the administration of chloral and butylchloral,[3] nitrobenzol,[4] orthonitrotoluol,[5] camphor,[6] etc. It also occurs in the urine after chloroform narcosis, and in the paralytic secretion that takes place on section of the renal nerves.[7] Occasionally it is found without any apparent cause, as a result of disordered metabolism.

Levulose on oxidation always yields acids containing less carbon atoms than itself, namely, trioxybutyric ($CH_2OH(CH.OH)_2COOH$), formic (H.COOH), and glycollic ($CH_2.OH.COOH$) acids. But different acids are yielded by different methods of oxidation; thus chlorine or bromine and silver oxide oxidise levulose to glycollic acid;[8] nitric acid yields oxalic, tartaric, glycollic, acetic, and other acids.[9]

Synthesis of sugars.—The first step towards the synthesis of the sugars was made by Butlerow.[10] He obtained a sugar-like substance by adding lime water to a solution of dioxymethylene; this he termed *methylenitan*, and gave its formula as $C_7H_{14}O_6$. Loew[11] next obtained a condensation product of formaldehyde (CH_2O) by means of lime water; to this substance he attributed the formula ($C_6H_{12}O_6$), and called it *formose*.

Neither methylenitan nor formose ferment with yeast. Fischer[12] investigated these substances, and found that they were mixtures of two sugars, one of which is formose ($C_6H_{12}O_6$), and another α-acrose[13] ($C_6H_{12}O_6$), both of which yield crystalline osazones.

From the osazone which is yielded by α-acrose the sugar can be again

[1] H. Thierfelder, *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 388; 1891, Bd. xv. S. 71; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1886, Bd. xix. S. 3148; E. Fischer and O. Piloty, *ibid.*, 1891, Bd. xxiv. S. 521.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ii. S. 47.

[3] Musculus and v. Mering, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xx. S. 64.

[4] v. Mering, *Centralbl. f. d. med. Wissensch.*, Berlin, 1875, No. 55.

[5] Jaffe, *loc. cit.*

[6] Schmiedeberg and Meyer, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 422.

[7] Ashdown, *Brit. Med. Journ.*, London, 1890, vol. i. p. 171.

[8] Hlasiwetz and Habermann, *Ann. d. Chem.*, Leipzig, Bd. clv.; Kiliani, *ibid.*, Bd. clv. S. 175.

[9] Kiliani, *ibid.*, S. 162; Hornemann, *Journ. f. prakt. Chem.*, Leipzig, Bd. lxxxix. S. 283.

[10] *Ann. d. Chem.*, Leipzig, Bd. cxx. S. 295; *Compt. rend. Acad. d. sc.*, Paris, tome lii. p. 145.

[11] *Journ. f. prakt. Chem.*, Leipzig, Bd. xxxiii. S. 321.

[12] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xix. S. 2133.

[13] Acrose is a sugar obtained by Fischer, *ibid.*, Bd. xx. S. 1093 and 2566, by acting on acrolein bromide with bases $(2C_3H_4OBr_2 + (2Ba(OH)_2 = C_6H_{12}O_6 + 2BaBr_2$; two isomeric sugars, α- and β-acrose are thus produced.

obtained by reduction through the intermediate *osone* (see p. 9). The sugar obtained is identical with levulose or fructose, except that it is optically inactive. If this inactive levulose (*i*-levulose) is submitted to the action of yeast, the levorotatory constituent (*d*-levulose) ferments, and the residue is dextrorotatory. This is *l*-levulose,[1] but it is not the natural sugar. The natural sugar was formed in the following way :—*a*-acrose was reduced to the corresponding alcohol, *a*-acrite, which is identical with *i*-mannite; from this the sugar *i*-mannose was obtained, which was fermented, and *l*-mannose alone remained. By further oxidation *i*-mannose yields *i*-mannonic acid. By fractional crystallisation of the morphine or strychnine salt of this acid, it can be separated into its two active (*d* and *l*) constituents, and from these the corresponding sugars (mannoses) are obtained by reduction, and these by means of the ozones into the corresponding levuloses, the *d*-levulose being the levorotatory natural sugar.

In order to get dextrose, the *d*- and *l*-mannonic acids are heated with quinoline; this partly decomposes these acids, yielding *d*- and *l*-gluconic acids, and by reduction of these acids the sugars *d*-glucose (or dextrose) and *l*-glucose are obtained.

Of the numerous sugars in the monosaccharide group, dextrose, levulose, and galactose possess special physiological interest.

Dextrose is found widely distributed in nature in grapes, and many other fruits; also in seeds and roots, and in honey. It is generally mixed with levulose. In the animal body it is the final result of the digestion of starch, and occurs in small quantities in the blood and lymph; traces only occur in normal urine. The quantity both in the blood and urine is increased in diabetes. It crystallises either in fine needles, free from water of crystallisation, or with 1 molecule of water of crystallisation in small plates; these melt at 100° and lose their water at 110° C. The water-free crystals melt at 146° and at 170° C. lose water, the residue being glucosane ($C_6H_{10}O_5$). By higher temperatures it is converted into caramel.

Dextrose is readily soluble in water; the solution is not so sweet as one of cane-sugar; it is dextrorotatory. The specific rotation[2] varies with temperature and concentration, but at 20° C. averages $+52°{\cdot}6$. A freshly-made solution may have nearly double this rotatory power, but on standing for some time, or on heating the solution, the rotation becomes normal. Dextrose is slightly soluble in cold, very soluble in hot alcohol. It is insoluble in ether.

Levulose is found with dextrose in the vegetable kingdom, and in honey. It is formed by the hydrolytic splitting of cane-sugar and other carbohydrates, but is obtainable with special ease from inulin. It is occasionally found in diabetic urine.[3] In many cases of diabetes it may be used with impunity in the food.

[1] The *l*, *i*, and *d* are prefixes primarily attached to isomeric sugars, to indicate their action on polarised light, which is due to the presence and position of an asymmetric carbon atom. The terms were introduced by Fischer to denote this character, but they have been extended to comprise derivatives of the original sugar, which derivatives may have the opposite rotatory power, as is seen in the above example, where a *d* sugar is levo- and an *l* sugar is dextrorotatory.

[2] The specific rotation $(\alpha)_D$ of any substance is the amount of rotation in degrees of a circle of the plane of polarised light, produced by 1 grm. of the substance dissolved in 1 c.c. of liquid, examined in a tube 1 decimetre long. It is measured for yellow (sodium) light.

[3] Leo (*Virchow's Archiv*, 1887, Bd. cvii. S. 108) has found as an occasional constituent of diabetic urine, a levorotatory sugar which is not levulose. Its reducing power is small,

Its crystals, which are difficult to obtain, are partly water-free ($C_6H_{12}O_6$) and partly contain water of crystallisation ($2C_6H_{12}O_6.H_2O$). Levulose is different in chemical constitution from the other sugars we have studied in this group. It, however, gives the same general tests; but its specific rotatory power has not been satisfactorily determined.

Galactose is obtained by the hydrolytic decomposition of lactose or milk-sugar, and from many other carbohydrates, especially gums and mucilages. It is obtained by the decomposition of a glucoside occurring in the brain called cerebrin.[1] It crystallises in needles or plates, which melt at 168° C. It is somewhat more difficult of solution in water than dextrose, and more strongly dextrorotatory.

Mannose or *seminose* is another monosaccharide which is of scientific interest, as it is the aldehyde of the alcohol (mannite) of which levulose is the corresponding ketone.

It does not occur free in nature. It is obtained from mannite by oxidation,[2] and also by the action of dilute sulphuric acid on the so-called reserve cellulose.[3]

Reactions of the monosaccharides.—(*a*) *Fermentation.*—They are directly fermentable by yeast into alcohol and carbonic acid ($C_6H_{12}O_6 = 2C_2H_5.OH + 2CO_2$); and by the *Bacterium lactis* into lactic acid ($C_6H_{12}O_6 = 2CH_3—CH.OH.—COOH$). But this property of fermentation is only possessed by those which occur in nature.

(*b*) *Reducing power.*—Being aldehydes or ketones, they are easily oxidisable, and reduce metallic oxides in alkaline media.

They cause a deposition of metallic silver in an ammoniacal silver solution containing some caustic soda; and of metallic bismuth from basic bismuth nitrate suspended in soda (Böttcher's test); and of the red cuprous oxide (Cu_2O), or of the yellow cuprous hydrate $Cu(OH_2)$, from an alkaline solution of cupric oxide, as in Trommer's and Fehling's tests.[4]

(*c*) When heated in the dry state, before they char, they yield a brownish product called caramel. A similar substance is formed by boiling with alkalies (Moore's test).[5] In the brown substance formed, among other bodies is *levulinic acid*, $CH_3—CO—CH_2—CH_2—COOH$.

its rotatory power weak ($\alpha_D - 26$); it forms an osazone. Neubauer and Vogel ("Anleitung zur qualitativen und quantitativen Analyse des Harns," 1890) suggest the name "laiose" for it.

[1] Thierfelder, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 209; Brown and Morris, *Proc. Chem. Soc.*, London, 1889, p. 167.

[2] Fischer and Hirschberger, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bde. xxi. S. 1805; xxii. S. 1155 and 3218. [3] Reiss, *ibid.*, Bd. xxii. S. 909 and 3218.

[4] In Allihn's method (*Journ. f. prakt. Chem.*, Leipzig, Bd. xxii. S. 55) of estimating the reducing power of a sugar, the cuprous oxide obtained by Fehling's method is collected and weighed as metallic copper. Pflüger (*Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. lxvi.) recommends that the cuprous oxide should be dried at 120° and weighed. O'Sullivan and Stern (*Journ. Chem. Soc.*, London, 1896, p. 1691), who have recently prepared dextrose from several sources, have found that 1 gr. of CuO is reduced by 0·4535 gr. of dextrose (1 gr. $Cu_2O = 0·5045$ gr. dextrose). On the relation between reducing power and specific rotation see a series of papers by H. T. Brown, G. H. Harris, and J. H. Millar (*Proc. Chem. Soc.*, London, 1896, pp. 241–244). If the reducing power of dextrose is taken as 100, that of levulose is 92 to 94 (*ibid.*, 1897, p. 4).

[5] F. Framm (*Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiv. S. 575) has found that Moore's test is accompanied by the formation of products of oxidation, namely aldehyde and formic acid.

(*d*) *Phenylhydrazine test.*—This is carried out in the following way:—

To 5 c.c. of a solution of sugar (which should not be stronger than 0·5 per cent.) 1 decigramme of phenylhydrazine hydrochloride and 2 decigrammes of sodium acetate are added, and the mixture heated on the water bath for half an hour. On cooling, if not before, a crystalline or amorphous precipitate separates out. If amorphous it may be dissolved in hot alcohol, the mixture diluted with water, and boiled to expel the alcohol, whereupon

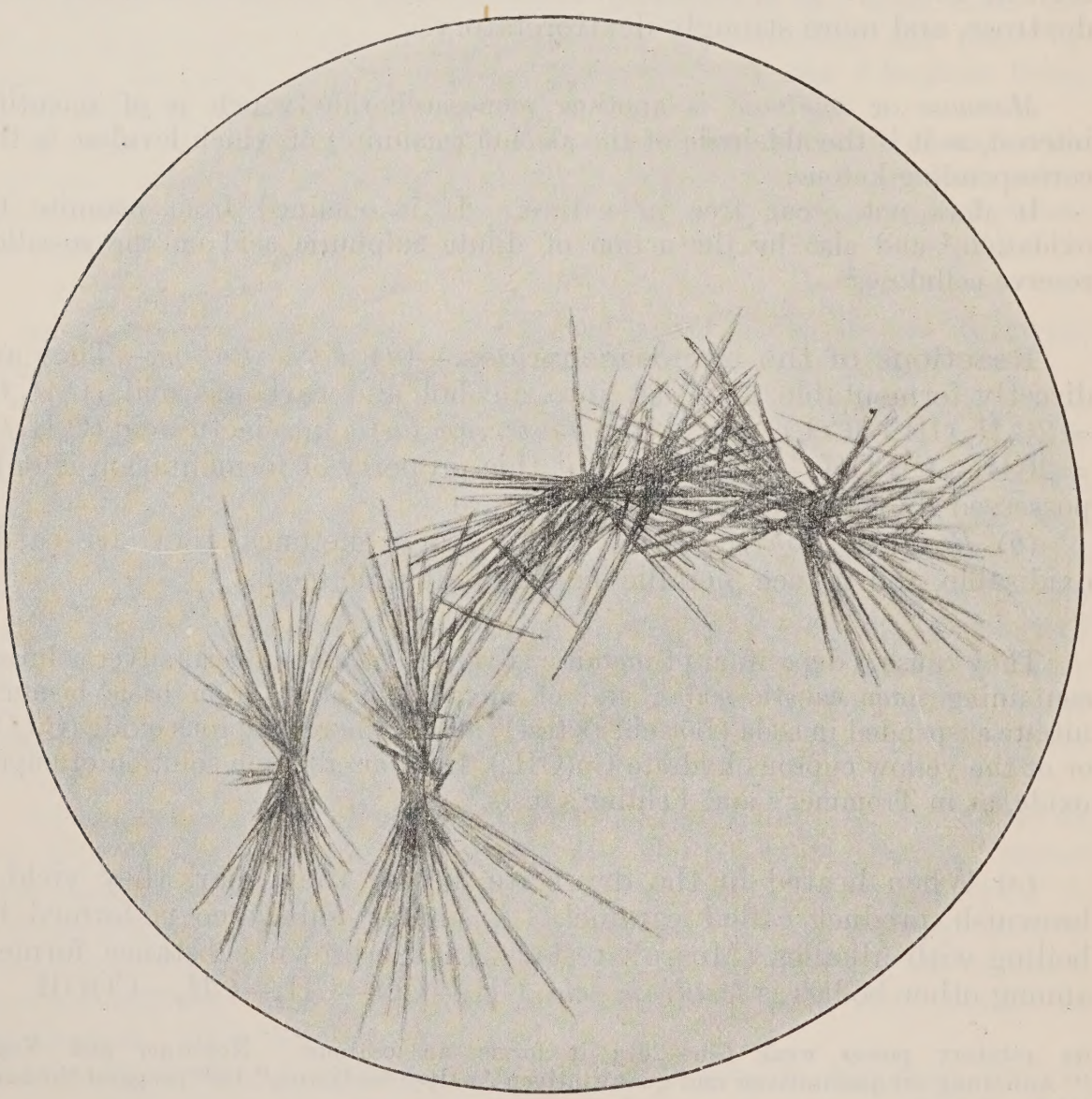


FIG. 1—Crystals of phenylglucosazone.

the compound or osazone separates out in yellow crystals. It is important that there should be an excess of phenylhydrazine.

Dextrose gives a precipitate of phenylglucosazone ($C_{18}H_{22}N_4O_4$), which crystallises in yellow needles (melting-point 205° C.). Levulose and mannose yield osazones identical with this.

Galactose yields a very similar osazone (phenylgalactosazone). It differs from phenylglucosazone by melting at 190–193° C., and in being optically inactive when dissolved in glacial acetic acid.

The chemistry of the reaction is represented in the following equations:—

1. $CH_2OH[CH(OH)]_3CH(OH)COH + H_2N.NH(C_6H_5)$
(dextrose) (phenylhydrazine)

$$= \begin{matrix} CH_2OH[CH(OH)]_3CH(OH)CH \\ \| \\ N—NH(C_6H_5) \end{matrix} + H_2O$$

(hydrazone) (water)

2. $CH_2OH[CH(OH)]_3CH(OH)CH$
$\|$
$N—NH(C_6H_5)$
(hydrazone)

$+ C_6H_5.NH—NH_2$
(phenylhydrazine)

$$= \begin{matrix} CH_2OH[CH(OH)]_3C—CH \\ \| \quad \| \\ C_6H_5.NH—N \quad N—NH.C_6H_5 \end{matrix} + H_2 + H_2O$$

(osazone) (hydrogen) (water)

The hydrogen seen in the second equation is not really set free, but it is used to split up a further molecule of phenylhydrazine into aniline and ammonia ($NH_2—NH.C_6H_5 + H_2 = NH_2C_6H_5 + NH_3$).

In order to obtain the sugar from the osazone again, it is first treated with fuming hydrochloric acid.[1] This gives rise to phenylhydrazine and a so-called *osone*. An osone is a substance which, besides the ketone group, contains an aldehyde group as well: $CH_2OH—(CH.OH)_3—CO—COH$.

By means of zinc and acetic acid the osone is easily reduced to sugar.

Glucosamine.—A derivative of glucose which is of some physiological interest is amido-glucose or glucosamine, $C_6H_{11}O_5.NH_2$. This is obtained on the decomposition of chitin and chondroitin. By treatment with nitrous acid it passes into dextrose—

$$C_6H_{11}O_5.NH_2 + NOOH = C_6H_{12}O_6 + N_2 + H_2O$$

Glucosamine can also be obtained by treating phenylglucosazone directly with reducing agents—

$$\underset{\text{(phenylglucosazone)}}{C_6H_{10}O_4.(N_2H.C_6H_5)_2} + H_2O + H_2 = \underset{\text{(glucosamine)}}{C_6H_{11}O_5.NH_2} +$$
$$\underset{\text{(phenylhydrazine)}}{NH_2—NH.C_6H_5} + \underset{\text{(aniline)}}{NH_2C_6H_5}$$

This shows us another way of regenerating the sugars from their osazones.[2] Further particulars about glucosamine will be found in connection with chitin and cartilage.

The disaccharides.—A disaccharide is a condensation product of two molecules of the simple sugars or monosaccharides, the change being attended with the loss of a molecule of water:—

$$C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O$$

Thus—

Cane-sugar is derivable from dextrose and levulose;
Milk-sugar, or lactose, from dextrose and galactose;
Maltose, from dextrose and dextrose.

The general properties of these sugars are like those of the monosac-

[1] E. Fischer, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1888, Bd. xxi. S. 2631; 1889, Bd. xxii. S. 87; 1890, Bd. xxiii. S. 2118.

[2] E. Fischer, *ibid.*, Berlin, Bd. xix. S. 1920; Fischer and J. Tafel, *ibid.*, Bd. xx. S. 2569.

charides; their solubilities are similar; they are optically active, crystallisable, diffusible, and sweet. Heated dry, they give rise to caramel. Further, they (with the exception of cane-sugar) reduce alkaline solutions of metallic oxides like Fehling's solution, and (again with the exception of cane-sugar) form crystalline osazones.

By hydrolysing agencies they take up water, and split into the simple sugars of which they are made up. Thus —

Cane-sugar + water = dextrose + levulose.
Maltose + water = dextrose + dextrose.
Lactose + water = dextrose + galactose.

Among the agents capable of producing this decomposition the *inverting* ferment of the small intestine must be particularly mentioned. The term *inversion* arose from the fact that, if cane-sugar is the substance acted on, the previously dextrorotatory solution becomes levorotatory, because the levorotatory power of the levulose is greater than the dextrorotatory power of the dextrose formed. The term inversion has, however, been extended to include the similar decompositions of lactose and maltose. The reverse action by which the monosaccharides are united to form disaccharides is called *reversion.*

Cane-sugar is generally distributed throughout the vegetable kingdom in the juices of plants and fruits, especially the sugar-cane, beetroot, mallow, and sugar-maple. As a food it is of high value. After abundant ingestion of cane-sugar, traces may be found in the blood and urine; but the greater part undergoes inversion in the alimentary canal.

It is readily soluble in water (100 parts of saturated solution contain 67 of sugar),[1] but soluble with difficulty in alcohol. It forms large, colourless monoclinic crystals. It is strongly dextrorotatory, and the amount of rotation does not vary so much with concentration and temperature as do most of the other sugars. The average value of $(\alpha)_D = +66{\cdot}5$.

Cane-sugar does not give many of the sugar tests; thus, it does not give Moore's test; with Trommer's test, it gives a blue solution, but no reduction occurs on heating. It does not react with phenylhydrazine, and it is not directly fermentable by yeast; the yeast, however, secretes an inverting ferment, and after inversion the glucoses formed are converted into alcohol and carbonic acid.

By boiling with concentrated hydrochloric acid a deep red solution is formed. Dextrose, maltose, and lactose do not give this reaction.

Maltose is one of the end products of the action of malt diastase on starch. It is also the chief sugar formed from starch by the diastatic ferments contained in the saliva and pancreatic juice. It is an intermediate product in the action of sulphuric acid on starch. It crystallises with one molecule of water of crystallisation in fine white needles. It is easily soluble in water and in alcohol; insoluble in ether. It is dextrorotatory; but its rotatory power decreases with concentration (relatively) and with rise of temperature.

For a 20 per cent. solution at 15° C. $(\alpha)_D = +139°{\cdot}3$. The amount of rotation is about 18° less for a freshly prepared solution than for one which has stood for some hours.[2]

[1] Scheibler, see Tollen's "Handbuch."

[2] Brown, Morris, and Millar (*Proc. Chem. Soc.*, London, 1896, p. 244) give $(\alpha)_D = +138°$.

Maltose reduces copper, bismuth, and other metallic salts in alkaline solutions, but its reducing power as measured by Fehling's solution is about one-third less than that of dextrose.[1] It does not reduce Barfoed's reagent[2] as dextrose does. It ferments readily with yeast. With phenylhydrazine, phenylmaltosazone is formed ($C_{24}H_{32}N_4O_9$); this crystallises in yellow needles much broader than those yielded by dextrose or lactose; it melts at 206° C. Unlike phenylglucosazone, it dissolves in seventy-five parts of boiling water, and is still more soluble in hot alcohol (Fig. 2).

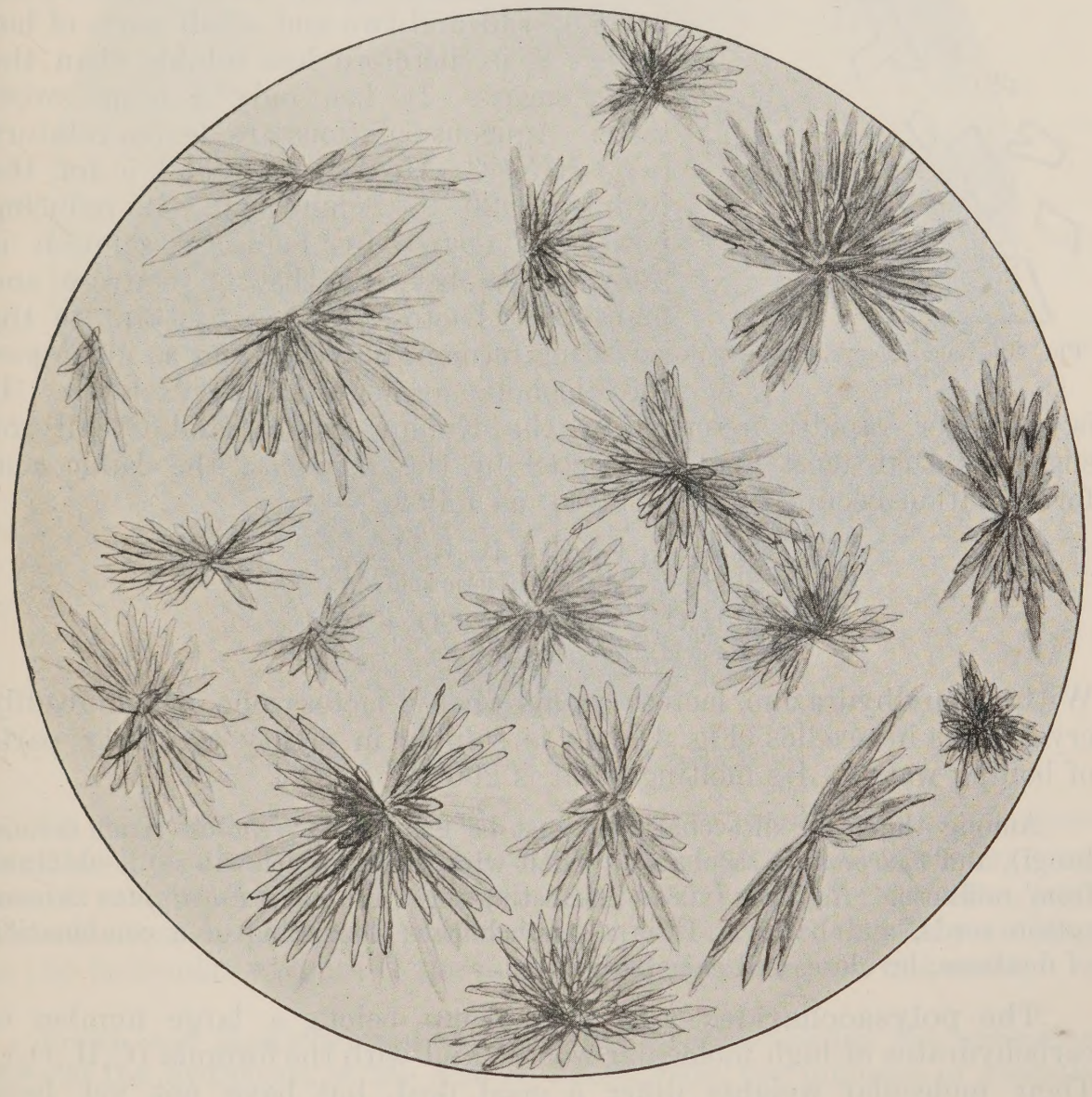


FIG. 2.—Crystals of phenylmaltosazone.

Isomaltose[3] is a sugar formed at the same time as maltose by the action of either diastase, ptyalin, or amylopsin[4] on starch. It is also an

[1] Ten c.c. of Fehling solution corresponds to 0·05 grms. of dextrose, levulose, or galactose, and to 0·07196 of maltose.

[2] 13·3 grms. of cupric acetate are dissolved in 200 c.c. of water; to this solution, 6 c.c. of acetic acid containing 38 per cent. of glacial acetic acid are added (Barfoed, "Organic Analysis," p. 254).

[3] Originally described by Fischer, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxiii. S. 3687. Fischer's observations, which have been called in question by some chemists, have been very generally confirmed. In his most recent paper on the subject, *ibid.*, 1896, Bd. xxvii. S. 3024, he shows that isomaltose is not directly fermentable by yeast, and so may be separated from maltose. Its osazone is soluble in four parts of hot water, while that from maltose requires seventy-five parts.

[4] Külz and Vogel, *Ztschr. f. Biol.*, München, Bd. xxxi.

intermediate product in the formation of dextrose by mineral acids from starch. An amylolytic ferment in blood serum, capable of forming dextrose from starch, acts similarly.[1] A small quantity occurs in normal urine.[2] It is readily soluble in water, is very sweet, and ferments slowly with yeast. Its general characters are like those of maltose, but its osazone forms fine yellow needles which melt at 150° C.

Lactose or milk-sugar occurs only in milk, and occasionally in the first days of lactation in the urine in small quantities.[3]

It crystallises in rhombic prisms, which contain one molecule of water of crystallisation (Fig. 3). It is soluble in six parts of cold, and two and a half parts of hot water; it is therefore less soluble than the other sugars. It has only a faint sweet taste. Aqueous solutions are dextro-rotatory $(\alpha)_D = +59°{\cdot}3$ (Hesse)[4] and $+52°{\cdot}5$ for the hydrate at 20° C. (Schmöger).[5] Its reducing power as tested by Fehling's solution is intermediate between that of dextrose and maltose.[6] Lactose is very resistant to the inverting ferment of yeast, and so undergoes the alcoholic fermentation very slowly. It is, however, rapidly inverted by the Kephir fungus, and of all the sugars is that most readily affected by the *B. lactis*; the lactic acid fermentation occurs in two stages, as follow:—

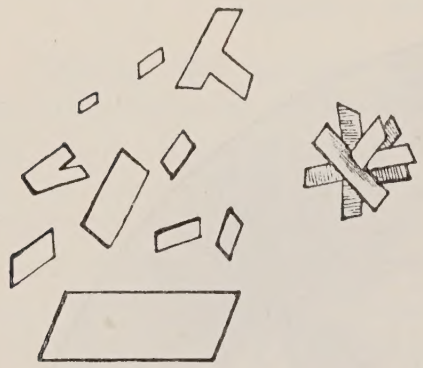


FIG. 3.—Lactose crystals.—After Frey.

$$1.\ \underset{\text{(lactose)}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{(lactic acid)}}{4C_3H_6O_3}$$

$$2.\ \underset{\text{(lactic acid)}}{2C_3H_6O_3} = \underset{\text{(butyric acid)}}{C_4H_8O_2} + 2CO_2 + 2H_2$$

With phenylhydrazine, lactose yields phenyl-lactosazone, which readily crystallises in needles (Fig 4). It is soluble in eighty to ninety parts of boiling water. Its melting point is 200° C.

Among the rarer disaccharides must be mentioned *trehalose* (from certain fungi), and *melebiose*, a saccharose which with *d*-fructose (levulose) is obtained from raffinose. *Raffinose*[7] is an interesting sugar found in *Eucalyptus manna*, cotton seeds, and barley. It is a trisaccharide, consisting of a combination of dextrose, levulose, and galactose.

The polysaccharides.—To this group belong a large number of carbohydrates of high molecular weight, and with the formula $(C_6H_{10}O_5)_n$. Their molecular weights differ a good deal, but have not yet been determined directly by chemical methods.[8] They are not crystalline, are indiffusible, and, as a rule, insoluble in cold water. In hot water they partially dissolve, forming opalescent solutions. Like the proteids,

[1] Röhmann, *Centralbl. f. d. med. Wissensch.*, Berlin, 1893, S. 849.

[2] Lemaire, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 442.

[3] The most recent observations on lactose in the urine of women after childbirth are by Lemaire, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 442. Pavy, *Lancet*, London, 1897, vol. i. p. 1075. See also Hofmeister, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. i. S. 101.

[4] *Ann. d. Chem.*, Leipzig, 1875, Bd. clxxvi. S. 98.

[5] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1880, Bd. xiii. S. 1922.

[6] Ten c.c. of Fehling's solution = 0·06334 lactose; see footnote 1, p. 11.

[7] Loiseau, *Compt. rend. Acad. d. sc.*, Paris, 1876, tome lxxxii. p. 1058; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. ix. S. 732; Scheibler, *ibid.*, 1886, Bd. xix. S. 2868.

[8] By Raoult's method of determining the lowering of the freezing point in very dilute solutions, Brown and Morris (*Journ. Chem. Soc.*, London, 1888, p. 610), have provisionally

they are precipitated from their solutions by saturation with certain neutral salts, such as ammonium sulphate.[1]

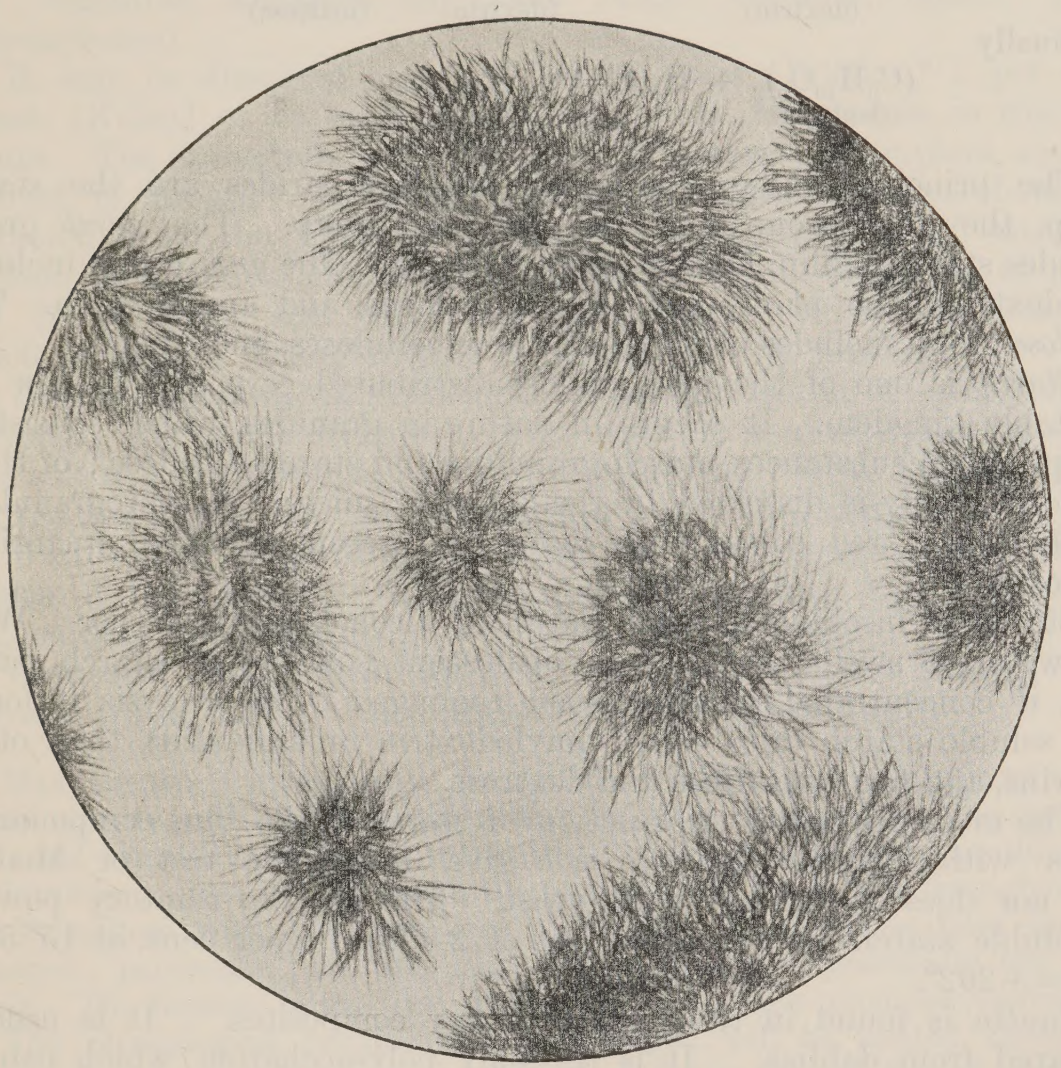


FIG. 4.—Crystals of phenyl-lactosazone.

By hydrolysis they are finally split up into simple sugars; various dextrins and disaccharides being intermediate products. The dextrins are of various kinds, and are differently named by different observers. The reaction cannot be represented by equations with certainty as long as the molecular weights of the members of the group are unknown.

Brown and Morris suggest the following series, indicating the successive steps of the hydrolysis, in the case of starch under the influence of diastatic ferments :—

$$\underset{\text{(starch)}}{(C_6H_{10}O_5)_n} + H_2O = \underset{\text{(dextrin)}}{(C_6H_{10}O_5)_{n-2}} + \underset{\text{(maltose)}}{C_{12}H_{22}O_{11}}$$

$$\underset{\text{(dextrin)}}{(C_6H_{10}O_5)_{n-2}} + H_2O = \underset{\text{(dextrin)}}{(C_6H_{10}O_5)_{n-4}} + \underset{\text{(maltose)}}{C_{12}H_{22}O_{11}}$$

$$\underset{\text{(dextrin)}}{(C_6H_{10}O_5)_{n-4}} + H_2O = \underset{\text{(dextrin)}}{(C_6H_{10}O_5)_{n-6}} + \underset{\text{(maltose)}}{C_{12}H_{22}O_{11}}$$

assigned to dextrin and soluble starch the formulæ $(C_6H_{10}O_5)_{12}$ and $(C_6H_{10}O_5)_{30}$ respectively. The same method applied to starch, though not so satisfactorily, points to a molecular weight of between 20,000 and 30,000 ; that is, about three times greater than that of soluble starch. Sabanejeff, *Chem. Centr.-Bl.*, Leipzig, 1891, S. 10 ; *Journ. Russian Chem. Soc.*, vol. xxi. p. 515, by the same method assigns to glycogen the formula $(C_6H_{10}O_5)_{10}$.

[1] Pohl, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 151 ; Young, "Proc. Physiol. Soc.," Feb. 13, 1897, in *Journ. Physiol.*, Cambridge and London, vol. xxi.

and so on, until at last we get to

$$\underset{\text{(dextrin)}}{(C_6H_{10}O_5)_4} + H_2O = \underset{\text{(dextrin)}}{(C_6H_{10}O_5)_2} + \underset{\text{(maltose)}}{C_{12}H_{22}O_{11}}$$

and finally

$$\underset{\text{(dextrin)}}{(C_6H_{10}O_5)_2} + H_2O = \underset{\text{(maltose)}}{C_{12}H_{22}O_{11}}$$

The principal sub-groups of the polysaccharides are the starch group, the gum group, and the cellulose group. The *starch* group includes starch, inulin, lichenin, and glycogen. The *gum* group includes the dextrins, the plant gums and mucilages, and animal gum. The *cellulose* group includes cellulose, the hemicelluloses, and tunicin.

Starch is one of the most widely distributed carbohydrates in the vegetable kingdom. It occurs in nature in granules, which consist of two principal substances, starch-granulose and starch-cellulose; of these the former only is dissolved by the digestive juices. Erythrogranulose, which gives a red colour with iodine, is present in small quantities (Brücke).

Starch is insoluble in cold water, in alcohol, and in ether. With hot water it swells, forming an opalescent solution or starch paste. This, if concentrated, gelatinises on cooling. On hydrolysis it forms first soluble starch (also called amylodextrin or amidulin), then other dextrins, and finally maltose and dextrose.

The most characteristic reaction of starch is the blue compound it forms with iodine.[1] It does not give Trommer's test or Moore's test, nor does it ferment with yeast. The specific rotatory power[2] of soluble starch for concentrations of 2·5 to 4·5 per cent. at 15°·5 C., $(\alpha)_D = +202°$.

Inulin is found in the roots of many composites. It is usually prepared from dahlias. It is the only polysaccharide which can be obtained in a crystallised form, namely, as sphero-crystals which polarise light. It is readily soluble in warm water; by cooling the solution it is precipitated. By hydrolysis its final product is levulose.[3]

Lichenin is a polysaccharide occurring in Iceland moss, and certain algæ. It is insoluble in cold water, soluble in hot water, gives a yellow colour with iodine, is converted into glucose by hot dilute mineral acids, but is not affected by saliva or pancreatic juice.[4]

Glycogen.—This is a small but constant constituent of protoplasm, and of animal tissues generally. It is found in white blood corpuscles,[5] and in pus,[6] occasionally in diabetic urine,[7] but is specially abundant in

[1] E. Zander finds that the iodine reaction given by polysaccharides and by chitin varies considerably with the solvent used (*Arch. f. d. ges. Physiol.*, Bonn, 1897, Bd. lxvi. S. 545).

[2] Brown, Morris, and Millar, *loc. cit.*

[3] Kulz, "Beitr. z. Path. des Diabetes," Marburg, 1894, S. 130.; Worm-Müller, *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiv. S. 576; 1885, Bd. xxxvi. S. 172; F. Hofmeister, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxv. S. 240. On "Inulin as a Precursor of Glycogen," see Miura, *Ztschr. f. Biol.*, München, Bd. xxxii.; he obtained very inconstant results.

[4] Nilson, *Upsala Läkaref. Förh.*, vol. xxviii., quoted by Hammarsten, in "Physiol. Chem.," 3rd German edition, S. 67.

[5] Salomon, *Deutsche med. Wchnschr.*, Leipzig, 1877, Nos. 8 and 35; *Centralbl. f. Physiol.*, Leipzig, Bd. vi. S. 512; Huppert, *Centralbl. f. Physiol.*, Leipzig, Bd. vi S. 394.

[6] Salomon, *loc. cit.*

[7] Leube, *Virchow's Archiv*, Bd. cxiii. S. 391.

liver and muscle,[1] in embryonic tissues generally,[2] and in the bodies of molluscs.[3] It has been described in pathological growths,[4] and in the vegetable kingdom in many fungi[5] (truffles, mucor, yeast, myxomycetes).

It may be dissolved out with boiling water (Brücke),[6] 2 per cent. potash (Külz),[7] or by trichloracetic acid,[8] from the tissues in which it occurs. The extraction with this acid is, however, incomplete, and the product is impure.[9] Huizinga[10] recommends that glycogen should be extracted from the liver by a mixture of equal parts of saturated solution of mercuric chloride, and Esbach's reagent (10 grms. of picric and 20 of citric acid in a litre of water). From this solution, which is proteid free, glycogen is precipitable by alcohol.

The pure material is a white tasteless powder, soluble in water, forming a strongly opalescent solution. It is insoluble in alcohol and in ether. It is strongly dextrorotatory;[11] $(\alpha)_D = +196°{\cdot}63$. With Trommer's test it gives a blue solution, but no reduction occurs on boiling.

With iodine it gives a port-wine red colour, which easily distinguishes it from starch. Its precipitability by basic lead acetate distinguishes it from dextrin.

Prolonged boiling with water or boiling with dilute mineral acids converts it into sugar. The diastatic ferments act similarly.

Max Cremer[12] investigated the action of dilute acids on glycogen; he found glucose and isomaltose (identified by their osazones), but no maltose. Külz and Vogel[13] investigated the action of diastatic ferments; parotid saliva produced isomaltose and maltose in the proportion of 1 to 2 from liver-glycogen, and isomaltose with small amounts of maltose and dextrose from muscle-glycogen; pancreatic juice and malt diastase produced practically the same result. The ferment in the liver which acts on glycogen produces *dextrose.*

The physiological relationships of glycogen will be treated elsewhere. There is much controversy on the subject of the origin and fate of glycogen. There is, however, little doubt that it is chiefly a storage product from the carbohydrates of the food,[14] and that after death it is transformed into dextrose; the principal controversies of recent years have centred round the question whether glycogen normally leaves the liver in the hepatic blood as sugar (as

[1] Claude Bernard, *Compt. rend. Acad. d. sc.*, Paris, 1857, tome xliv. pp. 578 and 1325; xlviii. pp. 77, 683, 763 and 784; Hensen, *Virchow's Archiv*, 1857, Bd. xi. S. 395; O. Nasse, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 97.

[2] Claude Bernard, "Physiologie expér.," 1855, tome i. p. 241; iv. p. 44; Salomon, *Centralbl. f. d. med. Wissensch.*, Berlin, 1874, S. 738; Moriggia, *ibid.*, 1875, S. 186; v. Wittich, Hermann's "Handbuch," 1883.

[3] Bizio, *Ztschr. f. Chem.*, Leipzig, 1866, S. 222; Bernard, "Leçons sur les phénomènes de la vie," 1879, tome ii.; Krukenberg, "Vergl. physiol. Studien," 1880, Bd. ii. S. 52.

[4] Kühne, *Virchow's Archiv*, Bd. xxxii. S. 536; Sotnitschewski, *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 220.

[5] Kühne, "Lehrbuch der physiol. Chem.," 1868, S. 334; Reinke and Rodewald, "Studien ueber das Protoplasma," Berlin, 1881, S. 34, 54, and 169; Errera, *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, Bd. iv. S. 451.

[6] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1871, Bd. lxiii. S. 214.

[7] *Ztschr. f. Biol.*, München, 1886, Bd. iv. S. 191.

[8] Fränkel, *Arch. f. d. ges. Physiol.*, Bonn, Bde. lii. S. 125; lv. S. 378.

[9] Weidenbaum, *ibid.*, Bde. liv. S. 319; lv. S. 380.

[10] *Arch. f. d. ges. Physiol.*, Bonn, Bd. lxi.

[11] Huppert, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 137.

[12] *Ztschr. f. Biol.*, München, 1894, Bd. xxxi. S. 181. [13] *Ibid.*, S. 108.

[14] According to Voit and his pupils (*Ztschr. f. Biol.*, München, Bd. xxviii. S. 245), the liver forms glycogen only from dextrose and levulose, or from those carbohydrates which are converted into these sugars before they reach the liver.

Bernard originally taught), or is employed in the synthesis of fat and proteid (as Pavy holds).

Dextrin is the name given to a number of intermediate substances formed during the hydrolysis of starch; the principal varieties are erythrodextrin, which gives a red colour with iodine; achroödextrin, which does not; and maltodextrin, which has a lower molecular weight than these.[1] The dextrins are dextrorotatory (maltodextrin has an $(\alpha)_D = +174°{\cdot}5$). They are soluble in water, and insoluble in alcohol and ether. They give a blue solution with Trommer's test, but no reduction occurs on boiling.

Animal gum was discovered by Landwehr,[2] and resembles achroödextrin and glycogen in some of its properties. It is a decomposition product of mucin. When boiled with dilute sulphuric acid it yields a reducing but unfermentable sugar. Animal gum, like the vegetable gums, gives gelatinous precipitates with copper and iron salts.

Animal dextran is a gummy material, secreted by the *Schizoneura lanuginosa*, a gall-producing louse that attacks elms.[3]

Vegetable gums and mucilages include such substances as gum arabic, wood gum, etc., which are of subordinate physiological interest.

Cellulose is the name given to a number of carbohydrates which form the chief constituent of vegetable cell walls. In old cells, where it becomes very insoluble, it is called *lignin*. The celluloses are insoluble in cold and hot water, in alcohol, ether, and dilute acids and alkalies. A specific reagent for dissolving them is Schweitzer's reagent (a solution of cupric hydrate in ammonia).

With iodine and concentrated sulphuric acid they are turned blue; with nitric acid they yield nitroso-compounds of an explosive nature. Prolonged treatment with strong mineral acids leads to the formation of sugars; in some cases glucose, in others mannose, is formed. Schulze's mannoso-cellulose,[4] found in coffee and other seeds, is not a hemicellulose (see next paragraph). The celluloses are not acted upon by the digestive ferments proper; but they may be broken up in the intestine by bacteria into carbonic acid and methane.

Hemicelluloses are those varieties of cellulose which differ from the others by yielding monosaccharides by treatment with *dilute* mineral acids. The hemicellulose of yellow lupins yields galactose and arabinose; that of rye and wheat, arabinose and xylose; that of certain nuts, mannose.[5]

Tunicin is animal cellulose. It is the chief constituent of the test or outer investment of the tunicates.[6]

Cellulose has also been found in the animal kingdom in the skin of the silkworm,[7] and in the zoocytium of *Ophrydium versatile*.[8]

Inosite.—Inosite is a substance found in muscle and other animal tissues, and in many vegetables also. Its crystalline form is shown

[1] Recent papers on dextrin will be found in *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bde. xxiii. S. 3060; xxvi. S. 2930 (by Scheibler and Mittelmeier), and Bd. xxvi. S. 2533 (by Leubner and Doll).

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. viii. S. 119, 124.

[3] Liebermann, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xl. S. 454.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvi.

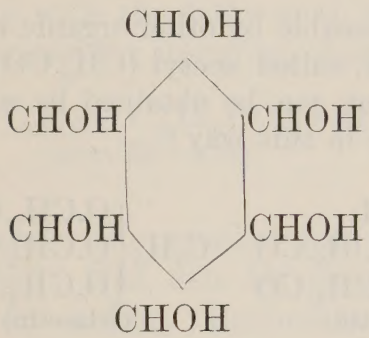
[5] See Schulze, *loc. cit.*; and Reiss, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxii.

[6] Schäfer, *Ann. d. Chem.*, Leipzig, Bd. ccx. S. 312; Berthelot, *Ann. de chim.*, Paris, Sér. 3, tome lvi. p. 153.

[7] De Lucca, *Compt. rend. Acad. d. sc.*, Paris, tome lii. p. 102; lvii. p. 43.

[8] Halliburton, *Quart. Journ. Micr. Sc.*, London, July 1885.

in Fig. 5. For many years it was regarded as a carbohydrate, though an exceptional one. It is sweet to the taste, but it gives none of the characteristic reactions of sugar. As the chemical constitution of the sugars was revealed, it became more and more evident that inosite is not a sugar. Its constitution was worked out by Maquenne[1] from a study of its nitro-substitution and other products. It belongs to the substances which have a closed carbon chain, and its graphic formula may be written thus:—



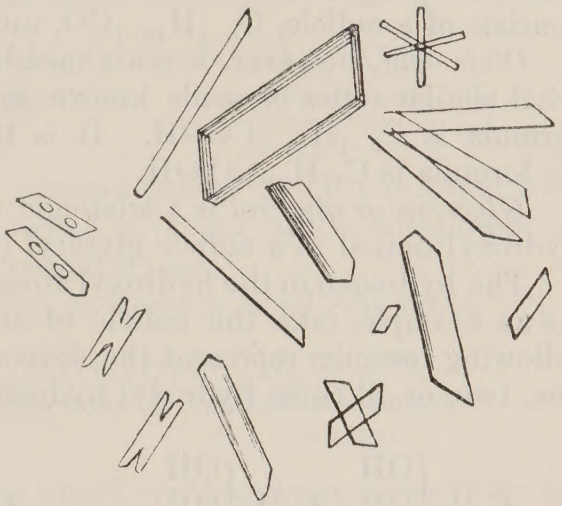


FIG. 5.—Inosite crystals.—After Frey.

THE FATS.

Fat is found in most of the animal tissues. The following table from Gorup-Besanez gives the percentage in the organs and fluids of the body:—

Sweat	0·001	Cartilage	1·3
Vitreous humour	0·002	Bone	1·4
Saliva	0·02	Crystalline lens	2·0
Lymph	0·05	Liver	2·4
Synovia	0·06	Muscles	3·3
Liquor amnii	0·06	Hair	4·2
Chyle	0·2	Brain	8·0
Mucus	0·3	Egg	11·6
Blood	0·4	Nerves	22·1
Bile	1·4	Adipose tissue	82·7
Milk	4·3	Marrow	96·0

The fats are usually extracted from the finely divided tissue by means of ether in a Soxhlet's apparatus, but in the case of many organs the extraction is incomplete. Dormeyer therefore recommends that the tissue should be subjected to artificial gastric digestion before the extraction with ether;[2] when this was done, flesh was found to yield an additional 0·75 per cent. of fat.

The fats are compounds of fatty acids with glycerin, and are termed glycerides or glyceric ethers. The fatty acids form a series of acids derived from the monatomic alcohols by oxidation; thus—

From methyl alcohol (CH_3HO) formic acid (H.COOH) is obtained.

From ethyl alcohol (C_2H_5HO) acetic acid (CH_3COOH) is obtained, and so on.

[1] *Compt. rend. Acad. d. sc.*, Paris, 1887, tome civ. pp. 225, 297, 1719, 1853. For colour reactions of inosite, see Scherer, *Ann. d. Chem.*, Leipzig, 1852, Bd. lxxxi. S. 375; Gaulois, *Ztschr. f. anal. Chem.*, Wiesbaden, 1865, Bd. iv. S. 264; Seidel, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1887, Bd. xx. S. 320.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lxi. S. 341; 1896, Bd. lxv. S. 90; F. N. Schulze (*ibid.*, Bd. lxv. S. 299; 1897, lxxii. S. 145) has used the same method for the extraction of the fat of blood, and numerous organs.

Or, in general terms—

From the alcohol with formula $C_nH_{2n+1}.HO$ the acid with formula $C_{n-1}H_{2n-1}.CO.OH$ is obtained. The sixteenth term of this series has the formula $C_{15}H_{31}.CO.OH$, and is called *palmitic acid*; the eighteenth has the formula $C_{17}H_{35}.CO.OH$, and is called *stearic acid*. Each acid, as will be seen, consists of a radicle, $C_{n-1}H_{2n-1}CO$, united to hydroxyl (HO).

Oleic acid, however, is not a member of this series, but belongs to a somewhat similar series of acids known as the acrylic series,[1] of which the general formula is $C_{n-1}H_{2n-3}COOH$. It is the eighteenth term of the series, and its formula is $C_{17}H_{33}.CO.OH$.

Glycerin or *glycerol* is a triatomic alcohol, $C_3H_5(OH)_3$—*i.e.* three atoms of hydroxyl united to a radicle glyceryl (C_3H_5).

The hydrogen in the hydroxyl atoms is replaceable by other organic radicles. As an example, take the radicle of acetic acid, called acetyl ($CH_3.CO$). The following formulæ represent the derivatives that can be obtained by replacing one, two, or all three hydroxyl hydrogen atoms in this way:—

$C_3H_5\{$							
OH	OH	OH	OH	OH	O.CH_3.CO	O.CH_3.CO	O.CH_3.CO
OH	OH	O.CH_3.CO	O.CH_3.CO	O.CH_3.CO	O.CH_3.CO	O.CH_3.CO	O.CH_3.CO
(glycerin)		(monoacetin)		(diacetin)		(triacetin)	

The contents of the fat cells of adipose tissue in man are fluid during life, the normal body temperature being higher than the melting point of the mixture of fats found there; but this is not the case in all (even warm-blooded) animals, for beef fat melts at about 45° C., and mutton fat at a still higher temperature. Human fat consists of the three glycerides—palmitin, stearin, and olein. They differ in chemical composition, melting point, and solubilities. Olein melts at −5° C., palmitin at 45° C., and stearin at 53° to 66° C. It is thus olein which holds the other two dissolved at the body temperature. All are soluble in hot alcohol, ether, and chloroform, but insoluble in water.

The proportion in which these fats are mixed differs in different animals; in cold-blooded animals olein is much more abundant than in warm-blooded animals. Human fat contains from 67 to 80 per cent. of olein. Mixed with these neutral fats, there is generally a small amount of free fatty acids.

Fats are also found in the vegetable kingdom, especially in seeds and fruits, but in many cases in the roots also.

Stearin, palmitin, and olein ought more properly to be called tristearin, tripalmitin, and triolein respectively. Each consists of glycerin, in which the three atoms of hydrogen in the hydroxyls are replaced by radicles of the fatty acid. This is represented in the following formulæ:—

Acid.	Radicle.	Fat.
Palmitic acid $C_{15}H_{31}.COOH$	Palmityl $C_{15}H_{31}.CO$	Palmitin $C_3H_5(OC_{15}H_{31}.CO)_3$
Stearic acid . $C_{17}H_{35}.COOH$	Stearyl . $C_{17}H_{35}.CO$	Stearin . $C_3H_5(OC_{17}H_{35}.CO)_3$
Oleic acid . $C_{17}H_{33}.COOH$	Oleyl . $C_{17}H_{33}.CO$	Olein . $C_3H_5(OC_{17}H_{33}.CO)_3$

[1] Acrylic acid itself ($C_3H_4O_2$) is obtained by the oxidation of acrolein (C_3H_4O), the aldehyde of allyl alcohol.

Under the influence of superheated steam, mineral acids, and in the body by means of certain ferments (for instance, the fat-splitting ferment of the pancreatic juice), a fat combines with water and splits into glycerin and the fatty acid. The following equation represents what occurs in a fat, taking tripalmitin as an example:—

$$\underset{\text{(palmitin—a fat)}}{C_3H_5(O.C_{15}H_{31}CO)_3}+3H_2O=\underset{\text{(glycerin)}}{C_3H_5(OH)_3}+\underset{\text{(palmitic acid—a fatty acid)}}{3C_{15}H_{31}CO.OH}$$

Saponification.—In the process of saponification much the same sort of reaction occurs, the final products being glycerin and a compound of the base with the fatty acid, which is called a soap.

Suppose, for instance, that potassium hydrate is used, we get—

$$\underset{\text{(palmitin—a fat)}}{C_3H_5(O.C_{15}H_{31}CO)_3}+3KHO=\underset{\text{(glycerin)}}{C_3H_5(OH)_3}+\underset{\text{(potassium palmitate —a soap)}}{3C_{15}H_{31}CO.OK}$$

To separate the neutral fats from one another, they have always to be saponified; this can be accomplished by potassium hydrate, or still better by sodium alcoholate (Kossel, Obermüller, and Krüger).[1] On evaporation of the alcohol, the soaps are dissolved in water, and precipitated by sugar of lead; the lead compound of oleic acid is soluble in ether; the remaining soaps are treated with soda on the water bath, dried, dissolved in alcohol, and separated by fractional precipitation with barium acetate or barium chloride.

In the decomposition of fat, propionic, acetic, and formic acids may be found, which are absent from the fat in the fresh condition. This occurs when the fat becomes rancid, and is also produced by putrefactive organisms in the alimentary canal. The process is one of oxidation, and the way in which lower terms of the series are produced may be illustrated by the following equations:—

$$\underset{\text{(propionic acid)}}{C_3H_6O_2}+O_3=\underset{\text{(acetic acid)}}{C_2H_4O_2}+CO_2+H_2O.$$

$$\underset{\text{(acetic acid)}}{C_2H_4O_2}+O_3=\underset{\text{(formic acid)}}{CH_2O_2}+CO_2+H_2O.$$

$$\underset{\text{(formic acid)}}{2CH_2O_2}+O_2=2CO_2+2H_2O.$$

Emulsification.—Another change that fats undergo in the body is very different from saponification. It is a physical rather than a chemical change; the fat is broken up into very small globules, such as is seen in the natural emulsion—milk.

The fats of *milk* resemble in a general way those of adipose tissue, but there is a considerable admixture of glycerides lower in the series (see "Milk").

The fats of *marrow* are also like those of adipose tissue. As will be noticed in the table on p. 17, bone marrow is the tissue which is richest of all in fat.

Eylert[2] described a new fatty acid in the marrow of ox-bone which he called medullic acid, but this was shown by Mohr[3] to be only stearic acid.

[1] Numerous papers in vols. xiv., xv., and xvi. of *Ztschr. f. physiol. Chem.*, Strassburg.
[2] *Vrtljschr. f. prakt. Pharmakol.*, Bd. ix. S. 330.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xiv. S. 390.

Mohr gives the proportion of the acids in marrow fat as—palmitic acid, 22; stearic acid, 10; and oleic acid, 63 per cent.

Among the exceptional forms of fat are the following:—

Spermaceti, obtained from the sperm whale. This fat sets into a solid, white crystalline mass, melting at from 30° to 50° C. Its chief constituent is the palmitate of cetyl alcohol, or ethal ($C_{16}H_{33}OH$). This alcohol is the one from which palmitic acid is derived in the same way as acetic acid is derived from ethyl alcohol. Spermaceti contains also small quantities of compounds of lauristic, myristic, and stearic acids, with the radicles of the alcohols lethal ($C_{12}H_{25}OH$), methal ($C_{14}H_{29}OH$), and stethal ($C_{18}H_{37}OH$).

Beeswax contains three chief constituents:—

(1) Myricin; this is its principal constituent; it is the palmitate of myricyl alcohol ($C_{30}H_{61}OH$); (2) Cerotic acid ($C_{27}H_{54}O_2$); and (3) Cerolein, which is probably a mixture of several substances.

Chinese wax is chiefly the cerotic acid compound of cerotyl alcohol ($C_{27}H_{35}OH$).[1]

Adipocere is the name given to a waxy substance which replaces the muscular tissue in corpses buried in damp soil, or which have been allowed to remain in water some time after death. It consists chiefly of the calcium soaps of palmitic and stearic acids, and in some cases of acid ammonium soaps also.[2] Hoppe-Seyler[3] considered that the change is the result of a ferment action.

Lipochromes, Lecithin, Cholesterin.

Lipochromes.—This name is given to the pigments which occur in fat and fatty tissues. They are mostly yellow or yellowish red. They include the pigment of the blood serum (serum lutein) and of the corpus luteum; the chromophanes or coloured oil globules of the retinal cones; the yellowish pigment in butter, adipose tissue, and egg-yolk; tetronerythrin, a reddish pigment, found in many invertebrates; and several vegetable pigments, such as carrotin, which is found in carrots and tomatoes. The lipochromes have been separated by their various solubilities after saponification; they give various colour reactions, such as a greenish-blue colour with iodine and sulphuric acid, and a green colour with nitric acid; they show absorption-bands towards the violet end of the spectrum, and especially in the region of the F line.

Nothing is known about their chemical constitution; carrotin, which has been examined more fully than the others, has been assigned the formula $C_{18}H_{24}O$ by Husemann, and $C_{26}H_{38}$ by Arnaud.[4]

[1] On these rarer forms of fat and wax, see Liebermann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1885, Bd. xviii. S. 1975.

[2] Quain, *Med.-Chir. Trans.*, London, 1850, p. 141; Virchow, *Verhandl. d. phys.-med. Gesellsch. in Würzburg*, Bd. iii.; Wetherill, *Journ. f. prakt. Chem.*, Leipzig, Bd. lxviii. S. 26; K. B. Lehmann, *Centralbl. f. Agric. Chem.*, Leipzig, 1889, S. 66.

[3] "Physiol. Chem.," Strassburg, S. 119. According to some authors, its formation is brought about by micro-organisms (Jacobsthal, *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 499.

[4] The principal papers on lipochromes are the following:—On lutein—Thudichum, *Centralbl. f. d. med. Wissensch.*, Berlin, 1869, Bd. vii. S. 1. On colour reactions of luteins—Thudichum, *loc. cit.*; Piccolo and Lieben, *Gior. de sc. nat. ed. econ.*, Palermo, vol. ii. p. 258; Caprarnica, *Arch. f. Physiol.*, Leipzig, 1877, S. 283.; Schwalbe, "Handb. d. ges. Augenheilkunde," Leipzig, 1874, Bd. i. S. 414. On chromophanes—Kühne and Ayres, *Journ. Physiol.*, Cambridge and London, 1878, vol. i. p. 109; *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, Bd. i. Heft 4. On

Lecithin is a complex fat of wide distribution. It is a constant constituent of protoplasm, and is found both in the animal and vegetable[1] world. In the animal tissues, it is found principally in the brain and nervous tissues, where it is probably a decomposition product of a more complex substance originally called protagon by Liebreich[2] (see section on "Chemistry of Nervous Tissues"); in yolk of egg;[3] and in blood corpuscles.[4] Lecithin is found in all organs composed of cells, and also in certain secretions, namely, semen, bile, and milk.

Lecithin is a yellowish white, waxy, hygroscopic solid, soluble in ether and in alcohol; it swells and forms a kind of emulsion with water. When ignited it burns and leaves a residue of metaphosphoric acid. Its most important compounds are those of its hydrochloride with platinum chloride $(C_{44}H_{90}NPO_9Cl)_2+PtCl_2$, and with cadmium chloride which has a corresponding formula.[5]

Montgomery[6] showed that when water, glycerin, and other reagents were added on a microscopic slide to impure lecithin (or protagon, as he termed it), prepared from egg-yolk, snake-like forms shoot out, bending and curling and even simulating nerve fibres or nerve cells. On cooling a solution of lecithin in alcohol, it separates out in crystalline clumps. On decomposition by alkalis, it yields glycero-phosphoric acid, a fatty acid, and an alkaloid choline.

Choline is an ammonium base, and has the following constitution:—

$$N\begin{Bmatrix}(CH_3)_3\\CH_2—CH_2OH\\OH\end{Bmatrix}=C_5H_{15}NO_2$$

It is therefore trimethyl-oxyethyl-ammonium hydroxide; its name is derived from the fact that it was first separated from the lecithin of the bile. Its synthesis was accomplished by Wurtz[7] from ethylene

tetronerythrin—Wurm, *Ztschr. f. wissensch. Zool.*, Leipzig, 1871, Bd. xxxi. S. 535; Merejkowski, *Compt. rend. Acad. d. sc.*, Paris, 1881, tome xciii. p. 1029; MacMunn, *Proc. Birmingham Phil. Soc.*, vol. iii. p. 351; *Proc. Roy. Soc. London*, 1883, No. 226, p. 17; Halliburton, *Journ. Physiol.*, Cambridge and London, 1884, vol. vi. p. 324; Krukenberg, *Centralbl. f. d. med. Wissensch.*, Berlin, 1879, Bd. ix. S. 705. On serum lutein—Krukenberg, *Sitzungsb. d. Jenaisch. Gesellsch. f. Med. u. Naturw.*, 1885; Halliburton, *Journ. Physiol.*, Cambridge and London, 1885, vol. vii. p. 324. On saponification of lipochromes—Kühne, *loc. cit.*; Maly, *Monatsh. d. Chem.*, Wien, 1881, Bd. ii. S. 351; Bein, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1890, Bd. xxiii. S. 204. On carrotin—Husemann, *Ann. d. chem.*, Leipzig, Bd. cxvii. S. 200; Arnaud, *Compt. rend. Acad. d. sc.*, Paris, tome cii. p. 119; civ. 1293. Newbiggin, "On Crustacean Pigments," *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 237.

[1] On the subject of lecithin and choline in vegetable oils, etc., see Heckel and Schlagdenhauffen, *Compt. rend. Acad. d. sc.*, Paris, tome ciii. p. 188; Jacobson, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxiii. S. 33; Schulze, *ibid.*, Bde. xi. S. 365; xii. S. 441; xvii. S. 204; J. Stoklasa, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1896, Bd. xxix. S. 2761.

[2] *Ann. d. Chem.*, Leipzig, Bd. cxxxiv. S. 29.

[3] Gobley, *Journ. de pharm. et chim.*, Paris, tomes xi., xii., xvii., xviii.; Parke, Hoppe-Seyler's "Med. Chem. Untersuch.," Berlin, Heft 2, S. 213; Hoppe-Seyler, *ibid.*, S. 215; Diaconow, *ibid.*, S. 221; *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, S. 2.

[4] Gobley, *Journ. de pharm. et chim.*, Paris, tome xxi. p. 250; Hermann, *Arch. f. Anat. u. Physiol.*, Leipzig, 1866, S. 33; Hoppe-Seyler, "Med. Chem. Untersuch.," Berlin, Heft 1, S. 140; Jüdell, *ibid.*, Heft 3, S. 386.

[5] The formation of these compounds enables one to prepare lecithin in a pure form, the metal being subsequently got rid of by sulphuretted hydrogen.

[6] "On the Formation of So-called Cells," London, 1867.

[7] *Ann. d. Chem.*, Leipzig, 1868, Supplement, Bd. vi. S. 116 and 197; see also Bayer, *ibid.*, Bd. cxl. S. 306.

oxide (C_2H_4O), trimethylamine $N(CH_3)_3$, and water. It was at one time thought to be identical with the base *neurine*, which Liebreich separated from nervous tissues, and the two are closely related; empirically choline ($C_5H_{15}NO_2$) is neurine ($C_5H_{13}NO$), plus water. In constitution neurine is trimethylvinylammonium hydroxide.

Glycero-phosphoric acid is glycerin, in which one of the hydroxyl hydrogens is replaced by phosphoric acid, less hydroxyl; thus—

HO		OH
C_3H_5HO	$(H_2PO_3)HO$	C_3H_5OH
HO		$O—PO_3H_2$
(glycerin)	(phosphoric acid)	(glycero-phosphoric acid)

If the other two hydroxyl hydrogens are replaced by the radicle of stearic acid, we obtain

$$\begin{array}{l} CH_2.O—C_{17}H_{35}CO \\ CH.O—C_{17}H_{35}CO \\ CH_2.O—PO\begin{cases} OH \\ OH \end{cases} \end{array}$$

which is distearyl-glycero-phosphoric acid. This is then united to choline (less hydroxyl), and we obtain lecithin, or distearyl lecithin, as it should be more properly termed; for other lecithins exist in which palmityl, oleyl, or other fatty acid radicles take the place of stearyl.

The exact manner of the union of the acid with choline is a matter of controversy, for up to the present lecithin has not been prepared synthetically. Hundeshagen [1] prepared artificially a choline salt of distearyl-glycero-phosphoric acid, which is isomeric with lecithin, but which possesses none of its characteristic properties.

The constitution of lecithin is not therefore that of a salt in which choline plays a part of the base, as Diaconow [2] first suggested, but more probably it is an ether-like combination, the choline radicle being united to the acid by means of the oxygen of the hydroxyl; the formula for distearyl-lecithin would therefore be (Strecker) [3]—

$$\begin{array}{l} CH_2.O—C_{17}H_{35}CO \\ CH.O—C_{17}H_{35}CO \\ CH_2.O—PO—O.C_2H_4\Big) \\ \qquad\quad | \qquad (CH_3)_3\Big\} N \\ \qquad OH \quad HO \quad\Big) \end{array}$$

The following equation represents the decomposition of lecithin, such as occurs on boiling it with alkaline solutions:—

$$\underset{\text{(lecithin)}}{C_{44}H_{90}NPO_9} + 3H_2O = \underset{\text{(stearic acid)}}{2C_{18}H_{36}O_2} + \underset{\text{(glycero-phosphoric acid)}}{C_3H_9PO_6} + \underset{\text{(choline)}}{C_5H_{15}NO_2}$$

Lecith-albumins.—See p. 69.

Cholesterin.—Cholesterin is contained in small quantities in all protoplasmic structures; it is also found in blood corpuscles and in bile. It is a large constituent of sebum and similar oily secretions of the skin. In nervous tissues it is an especially abundant constituent of the white substance of the medullary sheath. It may be prepared by making a hot

[1] *Journ. f. prakt. Chem.*, Leipzig, 1883, Bd. xxviii. S. 219; see also E. Gilson, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 585.

[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1868.

[3] *Ann. d. Chem.*, Leipzig, 1868, Bd. cxlviii. S. 77.

alcoholic extract of the brain or spinal cord; on cooling, the cholesterin, together with protagon and cerebrin, separates out. From this mixture the cholesterin is dissolved out with ether, and the ether distilled off. To get rid of traces of lecithin it is heated for an hour with alcoholic potash; this decomposes the lecithin, and the residue obtained by evaporating to dryness is dissolved in a mixture of alcohol and ether; from this solution, cholesterin crystallises out as its solvents evaporate off.

Cholesterin is readily obtained from gall stones by simply extracting them with boiling alcohol, and treating with alcoholic potash to free it from extraneous matter.

Like the fats, cholesterin is insoluble in water, but freely soluble in hot or cold ether, glycerine, benzol, hot alcohol, and chloroform. From anhydrous ether or chloroform it separates in the form of needles, containing no water of crystallisation; from alcohol, or ether containing water, it separates in the form of rhombic, bright tables, which contain a molecule of water of crystallisation, and are easily identified by the microscope (Fig. 6).

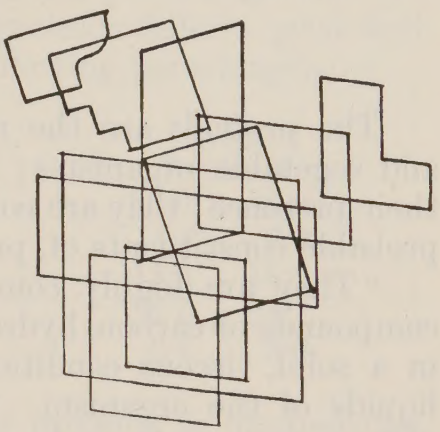


FIG. 6.– Cholesterin crystals.—After Frey.

Dry cholesterin melts at 145°, distils *in vacuo* at 360° C.; its specific rotatory power is $(\alpha)_D = -31°{\cdot}6$. It may be recognised by the following colour tests:—

1. With iodine and concentrated sulphuric acid the crystals give a play of red, green, and blue.

2. *Salkowski's reaction.*[1]—The cholesterin is dissolved in chloroform and an equal volume of concentrated sulphuric acid added; the solution becomes first red and then purplish, while the sulphuric acid is dark red with a green fluorescence. On pouring off the chloroformic solution, it becomes green and finally yellow.

3. *Liebermann-Burchard's reaction.*[2]—This is a very delicate test, and is stated to be capable of detecting one part of cholesterin in 20,000 of solvent. The cholesterin is dissolved in 2 c.c. of chloroform, and ten drops of acetic anhydride are added, and then concentrated sulphuric acid drop by drop. The mixture becomes first red, then blue, and finally green.

Cholesterin is a monatomic alcohol, the formula of which has been given as $C_{26}H_{43}OH$ and $C_{27}H_{45}OH$. The second formula was first ascribed to the substance by Reinitzer,[3] and it is probably the correct one, as it has been confirmed by the careful work of Obermüller.[4] These observers have prepared a large number of compounds and derivatives of cholesterin, but its constitution still remains unknown.

The subject is complicated by the circumstance that there are several isomeric cholesterins.

[1] *Arch. f. d. ges. Physiol.*, Bonn. Bd. vi.

[2] Liebermann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xviii. S. 1804; Burchard, "Beiträge zur Kenntniss der Cholesterine," Rostock, 1889.

[3] Reinitzer, *Monatsh. f. Chem.*, Wien, 1888, Bd. ix. s. 421.

[4] *Arch. f. Physiol.*, Leipzig, 1889; *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bde. xv. S. 37; xvi. S. 143, 152.

It forms, like glycerine, compounds often called *esters*, with fatty acids; and these compounds, which are found in the fatty secretions of the skin, especially in the fat of sheep's wool (lanoline), are very resistant to bacterial action; as a protection to the skin lanoline is therefore admirable.

In lanoline there are two cholesterins at least; one is levorotatory, the other (isocholesterin) is dextrorotatory. Isocholesterin was first described by Schultze,[1] and does not give Salkowski's reaction.

Cholesterins of various kinds are present in vegetable tissues.[2]

The cholesterin of the blood is in combination with oleic and palmitic acids.[3]

In man the cholesterin of the bile passes away in the fæces as koprosterin ($C_{27}H_{48}O$); in the horse as hippokoprosterin ($C_{27}H_{54}O$ or $C_{27}H_{56}O$); in the dog it is unchanged.[4]

The Proteids.[5]

The proteids are the most important substances present in animal and vegetable organisms; none of the phenomena of life occur without their presence; they are constant decomposition products of, and therefore probable constituents of, protoplasm.

"They are highly complex and, for the most part, uncrystallisable compounds of carbon, hydrogen, oxygen, nitrogen, and sulphur,[6] occurring in a solid, viscous condition, or in solution in nearly all the solids and liquids of the organism. The different members of the group present differences in physical, and to a certain extent even in chemical properties. They all possess, however, certain common chemical reactions, and are united by a close genetic relationship" (Gamgee).[7]

The following table from Gorup-Besanez[8] exhibits the percentage of proteids contained in the liquids and solids of the body:—

Cerebro-spinal fluid	0·09	Chyle	4·09
Aqueous humour	0·14	Blood	8·56
Liquor amnii	0·70	Spinal cord	7·49
Intestinal juice	0·95	Brain	8·63
Pericardial fluid	2·36	Liver	11·64
Lymph	2·46	Thymus	12·29
Pancreatic juice	3·33	Muscle	16·18
Synovia	3·91	Tunica media of arteries	27·33
Milk	3·94	Crystalline lens	38·30

[1] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. vi.; *Journ. f. prakt. Chem.*, Leipzig, N.F., Bd. xxv. S. 458; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 522. On isocholesterin in *vernix caseosa* see Rappel, *ibid.*, Bd. xxi. S. 122.

[2] Beneke, *Jahresb. ü. d. Leistung. . . . . d. ges. Med.*, Berlin, 1862; Hesse, *Ann. d. Chem.*, Leipzig, Bd. cxcii. S. 177; Bd. ccx., S. 283; Reinke and Rodewald, *ibid.*, Bd. ccvii. S. 232; Schulze and Barbieri, *Journ. f. prakt. Chem.*, Leipzig, N.F., Bd. xxv. S. 159, 458; Heckel and Schlagdenhauffen, *Compt. rend. Acad. d. sc.*, Paris, 1886, tome cii. p. 1317; Arnaud, *ibid.*, p. 1319. See also Jacobson's paper on "Vegetable Oils," *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 32.

[3] K. Hürthle, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 331.

[4] St. Bondzynski and V. Humnicki, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 396.

[5] In the preparation of this section I have derived special assistance from the articles "Eiweisskörper," in Beilstein's "Handbuch der org. Chemie," and in Ladenburg's "Handwörterbuch d. Chemie," 1885, Bd. iii. S. 534 (article by E. Drechsel); and from an article by T. G. Brodie in *Science Progress*, London, 1895, vol. iv. p. 62.

[6] In some cases phosphorus also is present.

[7] "Physiological Chemistry," London, vol. i. p. 4.

[8] "Lehrbuch," S. 128.

The proteid constituents of the animal body are derived from vegetables either directly, or indirectly through the body of another animal. Synthetic processes do occur in the animal body,[1] but to a much greater extent in vegetables; here the proteids are built up from simpler compounds, derived ultimately from the soil and atmosphere. In animals, the proteids are converted during digestion into hydrated products, called peptones; these are re-converted into proteids, similar, in a general sense, to those originally ingested, and these are assimilated to become part of the living organism. In time, they become subjected to katabolic processes, and give rise to carbonic acid, sulphuric acid, water, and certain not fully oxidised products (urea, uric acid, etc.) which contain the nitrogen of the original proteid.

Composition of the proteids.—Various proteids differ a good deal in elementary composition, as is seen by the following percentages:—

	From Hoppe-Seyler.[2]	From Drechsel.[3]
C	51·5 to 54·5	50·0 to 55·0
H	6·9 „ 7·3	6·8 „ 7·3
N	15·2 „ 17·0	15·4 „ 18·2
O	20·9 „ 23·5	22·8 „ 24·1
S	0·3 „ 2·0	0·4 „ 5·0

In addition to the above constituents, many proteids or proteid-like substances contain small quantities of phosphorus; and practically all proteids leave on ignition a variable amount of ash. In the case of egg-albumin the chief substances in the ash are chlorides of potassium and sodium, and smaller quantities of phosphoric, sulphuric, and carbonic acids, in combination with sodium, potassium, calcium, magnesium, and iron. There may also be a trace of silica.[4] The ash of serum proteids contains an excess of sodium chloride, and that of muscle proteids a preponderance of potassium and phosphoric acid.

Whether these mineral substances are integral constituents of the proteid molecule, or closely adherent impurities, is a matter of doubt; the latter supposition is the more probable, as there are certain methods of obtaining proteids practically free from ash. The best of these is Harnack's,[5] in which he precipitates the proteid as a copper albuminate; this is dissolved in sodium hydrate, and the proteid is precipitated from this solution by hydrochloric acid. The so-called ash-free albumin obtained earlier by Aronstein and Schmidt[6] by means of dialysis, was shown by later observers (Heynsius, Winogradoff) to be poor in ash, but not free from ash, and, moreover, that its incoagulability by heat and other characteristic properties were due to the use of alkali in its preparation. Nevertheless, Harnack's ash-free albumin is also not coagulable by heat, and more closely resembles acid albumin in its properties than any other known proteid.[7]

[1] A very suggestive article by Pflüger on this subject will be found in *Arch. f. d. ges. Physiol.*, Bonn, Bd. xlii. S. 144.

[2] "Handbuch d. physiol. path. chem. Anal.," 1885, 5th edition, S. 258.

[3] *Loc. cit.* Kühne and Chittenden's analyses of peptones, which they give with reserve, lie considerably outside these limits, *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 452.

[4] Gmelin, "Handb. d. org. Chem.," Bd. viii. S. 285.

[5] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxii. S. 3046; Bd. xxiii. S. 3745; Bd. xxv. S. 204.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1875, S. 1.

[7] Werigo, *ibid.*, Bd. xlviii. S. 127. Harnack denies that his material is acid-albumin, in spite of the acid used in its precipitation.

Globin prepared from hæmoglobin is stated to be free from ash. It is perhaps hardly correct to say that the ash is an impurity, because it is extremely probable that in their native condition the actual proteid molecules are combined more or less loosely with inorganic substances.

The process of incinerating has its drawbacks in determining the amount of ash in a proteid; for in the heating, some of the sulphur of the proteid, and when phosphorus is present the phosphorus also, will be oxidised and form sulphuric and phosphoric acids respectively. H. Schulz[1] has recently shown that sulphates are formed in tissues as a result of drying them at 110° C.; this would occur to a greater extent still at the temperature necessary for ignition.

The sulphur in proteids is in the body normally burnt off as sulphuric acid, which leaves the body in the urine as sulphates. The ethereal hydrogen sulphates of the urine originate in the intestine, as a result of putrefactive changes in proteids,[2] and when putrefaction is hindered by the administration of large doses of iodoform in dogs, these products do not appear in the urine.[3] Krüger[4] has shown that a part of the sulphur in proteids is present in a condition of stable combination, a part loosely combined; the latter is removed by boiling with alkalis, the former is not; the proportions of the two differ in different proteids. Among the primary decomposition products of proteid, thio-acids, of which thioglycollic acid is probably the most abundant, are obtained.[5]

From the elementary analyses which have been made of proteids, various observers have attempted to construct an empirical formula for certain typical proteids, egg-albumin being the one usually selected. Thus Lieberkühn assigned to albumin the formula $C_{72}H_{112}N_{18}O_{22}S$; Loew[6] gives the same formula; Harnack[7] gives $C_{204}H_{322}N_{52}O_{66}S_2$; Schützenberger,[8] $C_{240}H_{392}N_{65}O_{75}S_3$; and there have been others. The great divergence between these numbers requires no comment.

Equally conflicting results have been obtained in attempts to ascertain the molecular weight of albumin. Lieberkühn, in 1852, attempted to establish it by analysing the copper compound of egg-albumin; more recently, Harnack has done similar work. But very little importance can be attached to such work at present, for Chittenden and Whitehouse[9] find there is no definite copper albuminate, but that there are several in the mixture; and equally variable results are obtained with other metals both with egg albumin and myosin.

Such researches lead to the same conclusion as dialysis, namely, that the molecules of proteid are extremely large, but leave us quite in the dark as to their exact magnitude.[10] It is possible that in the future the

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lvi. S. 203. See also Halliburton and Brodie, *Journ. Physiol.*, Cambridge and London, 1894-95, vol. xvii. p. 154.

[2] Baumann, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. x. S. 123.

[3] Morax, *ibid.*, S. 318. See also more recently Nuttall and Thierfelder on "Animal Life without Bacteria in the Alimentary Canal," *ibid.*, vol. xxi. p. 109; xxii. p. 62. In this paper it is shown that healthy animal life is possible without micro-organisms in the alimentary canal.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. S. 244.

[5] F. Suter, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 564; E. Baumann, *ibid.*, S. 583, and *Virchow's Archiv*, 1894, Bd. cxxxviii. S. 560; E. Salkowski, *ibid.*, S. 562.

[6] Loew and Bokorny, "Die chemische Kraftquelle im lebenden Protoplasma," Munich, 1882.

[7] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. v. S. 207.

[8] *Bull. Soc. chim.*, Paris, Sér. 5, tomes xxiii. and xxiv. See also Schmiedeberg, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1897, Bd. xxxix. S. 1.

[9] *Stud. Lab. Physiol. Chem.*, New Haven, vol. ii. p. 95.

[10] The large size of the proteid molecule can be very strikingly demonstrated by the fact

result will be achieved, when proteids obtainable in a crystalline form have been thoroughly investigated.

Vegetable proteids have been prepared in a crystalline form[1] in combination with magnesia; Drechsel[2] found in one preparation 1·4 per cent. of magnesia (MgO); in another, prepared by an improved method, 1·43 per cent. From this the molecular weight x may be calculated as follows:—

$$\frac{x}{40} = \frac{100 - 1{\cdot}43}{1{\cdot}43}; \; x = 2757$$

From the similar examination of the sodium compound the molecular weight of albumin was found to be 1496. Other vegetable proteids examined by Grübler[3] also gave high but variable molecular weights.

Hæmoglobin belongs to the proteid compounds capable of crystallisation; Zinoffsky[4] prepared hæmoglobin crystals from the blood of the horse in a very pure state, and the formula calculated for hæmoglobin from his elementary analyses would be—

$$C_{712}H_{1130}N_{14}O_{245}FeS_2$$

If a molecule of hæmatin, $C_{32}H_{32}N_4O_4Fe$, is subtracted, the formula for proteid left is—.

$$C_{680}H_{1038}N_{210}S_2O_{241}$$

Jaquet's[5] formula for pure hæmoglobin of dog's blood would give the proteid molecule a formula—

$$C_{726}H_{1171}N_{194}S_3O_{214}$$

So that here again there are great discrepancies.

Such a summary of the principal analyses made, is quite sufficient to give point to Drechsel's conclusion, that while divergences of analysis exist, even though they are due to extremely small errors, it is futile to attempt to measure accurately the size of the proteid molecule. Drechsel points out that in so large a molecule an analytical error of 0·01 per cent. would have the same importance as one of 0·1 per cent. in ordinary analyses.

It should be added, in conclusion, that some few investigators have used the cryoscopic method in attempting the solution of this problem; the molecular weight of egg-albumin by this method is 14,000 (Sabanejeff),[6] of albumoses 1200–2100, and of antipepton much less (Paal).[7]

Equally inconclusive, though much more interesting, have been the attempts to discover the rational formula for the proteid molecule. The

that proteids in solution will not pass through a membrane of gelatin or silicic acid, when filtered under pressure. The products of proteolysis (proteoses and peptones) will, however, pass such a membrane; the smaller size of their molecules has also been demonstrated by the cryoscopic method. Crystalloids pass through such membranes at the same rate as water, and can thus be easily separated from colloids in a solution containing both (C. J. Martin, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 364).

[1] The subject of vegetable and crystalline proteids will be treated at length in a later section of this chapter.

[2] *Journ. f. prakt. Chem.*, Leipzig, 1879, N.F., Bd. xix. S. 331.

[3] *Ibid.*, 1881, Bd. xxiii. S. 97.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. x. S. 16. [5] Inaug. Diss., Basel, 1889.

[6] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1891, Bd. xxiv. Ref. 558.

[7] *Ibid.*, 1894, Bd. xxvii. S. 1827. For Siegfried's work on the identity of antipeptone with a simple compound, which he has called *carnic acid*, see under "Chemistry of Muscle," p. 103.

usual method which a chemist follows in attempting to unravel the constitution of any substance, is first to discover the way in which it decomposes (analysis), and then to build up the original material once more from the simple compounds so obtained (synthesis). In the case of the proteids there have been many observations on the analytical side, but synthesis has not yet been successful. We will first consider the results of analysis, next the attempts at synthesis, and finally state some of the theories founded on these observations.

The decomposition products of proteids.—The experiments which have been performed fall into two main groups: the first, designed with a view to determine the series of changes a proteid undergoes in its passage through the body; the second, with the object of investigating the chemical substances obtained as cleavage products by artificial means in the laboratory. In the first group the progress which has been made is slight, great and obvious difficulties being encountered at nearly every step; the end products, carbonic anhydride, water, urea, uric acid, ammonia, etc., are known, but the intermediate substances, resulting from metabolic changes within the cells and tissues, are still in the region of conjecture.

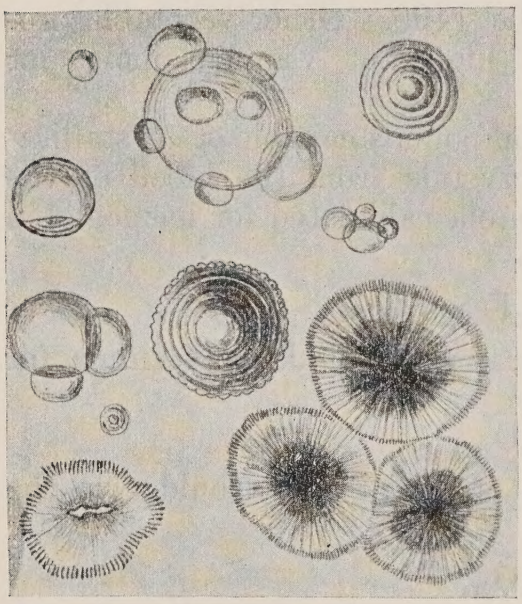


FIG. 7.—Leucine crystals.—After Kühne.

In the alimentary canal itself there are, however, changes which are within the grasp of the investigator, and the proteoses, albuminates, and peptones there formed will be treated under the head of "Digestion." Here, too, under the prolonged action of the pancreatic juice, simpler nitrogenous substances, such as leucine, tyrosine, aspartic acid, and ammonia, are formed in small quantities. Leucine ($C_6H_{13}N_2O$) is empirically amido-caproic acid, but of the numerous possible isomerides which could be included under that name, leucine has been shown to be α-amido-isobutylacetic acid, $(CH_3)_2CH.CH_2CH_2(NH_2)COOH$. The leucine obtained on pancreatic digestion is dextrorotatory. Levorotatory and optically inactive varieties of leucine exist, and some of them have been

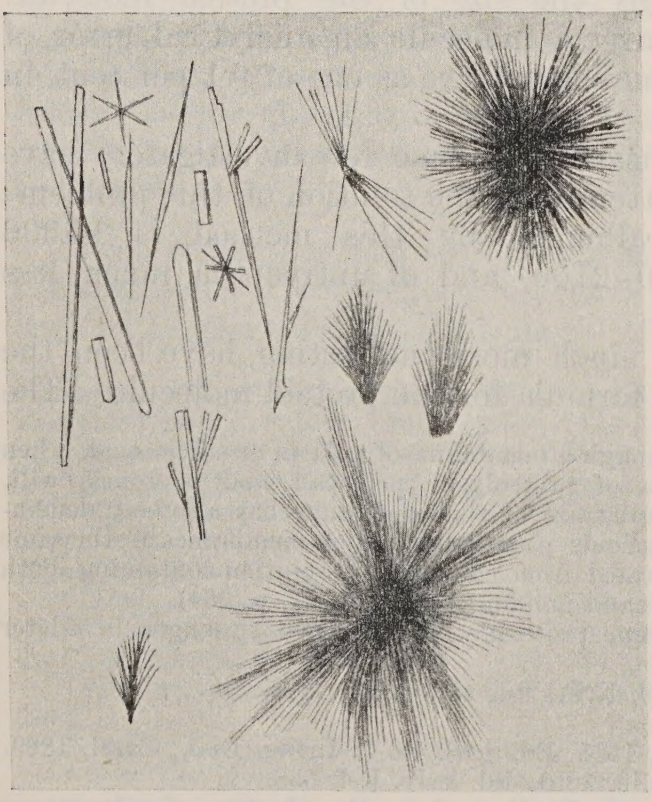


FIG. 8.—Tyrosine crystals.—After Frey.

prepared synthetically.[1] Tyrosine ($C_9H_{11}NO_3$) is oxyphenyl amidopropionic acid, $HO.C_6H_4.C_2H_3(NH_2).COOH$. This substance has also been made synthetically.[2] The crystalline forms of these two substances are seen in the accompanying figures (Figs. 7 and 8). Aspartic or asparaginic acid [3] ($C_4H_7NO_4$) is amido-succinic acid, $C_2H_3(NH_2)(COOH)_2$. That ammonia is produced in prolonged pancreatic digestion, under conditions precluding the possibility of putrefaction, was shown by Stadelmann.[4]

To this list must be added lysine, lysatinine, arginine[5] (see p. 33), glutaminic acid, and proteinchromogen,[6] a substance of uncertain nature which gives a reddish-violet product with chlorine or bromine water.

Within the intestine many changes occur which are due to bacterial action. The products which have just been enumerated arise first, and then by different changes other substances are formed; of these the following may be mentioned:—indol, skatol, skatol-carbonic acid, oxyphenylpropionic acid, phenylpropionic, and phenylacetic acids, parakresol, and phenol, and simpler bodies like carbonic anhydride, water, ammonia, hydrogen, and sulphuretted hydrogen, amido-fatty acids, and fatty acids themselves.[7] The most interesting point to note here is the large number of derivatives containing the benzene nucleus. The indol group has never been obtained from the proteid molecule by any other method than that of bacterial decomposition.[8]

We can now pass to the second category of investigations, namely, those carried out *in vitro*.

The first action produced by most reagents, especially if they bring about hydrolysis, is the formation of proteoses and peptones; these are then broken up into more simple substances. The subject may be most conveniently treated of under the heads of the different methods employed.

1. *Treatment with alkalis.*—Mulder [9] treated albumin with caustic potash, and obtained the substance which we now call alkali-albumin; this material is free from most of the sulphur present in the original proteid, namely, that which is present in loose combination; the firmly combined sulphur, however, remains undisturbed.[10]

Mulder thought that by this method he had obtained the base of all albuminous material, and called it "protein"; he described many

[1] For recent literature on leucine, see Schulze and Likiernik, *Ztschr. f. physiol. Chem.* Strassburg, Bd. xviii.; Gmelin, *ibid.*, Bd. xviii.; Hüfner, "Synthesis of Leucine," *Journ. f. prakt. Chem.*, Leipzig, N. F., Bd. i.; E. Schulze, Barbieri and Bosshard, *Ztschr. f. physiol. Chem.*, Strassburg, Bde. ix. and x.; Cohn, *ibid.*, Bd. xx.

[2] Erlenmeyer and Lipp, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xv. S. 1544.

[3] For chemistry and preparation, see Hlasiwetz and Habermann, *Ann. d. Chem.*, Leipzig, Bd. clxix. S. 160; E. Schulze, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ix.

[4] *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 261. See also Hirschler, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. x. S. 302.

[5] Hedin, *Arch. f. Physiol.*, Leipzig, 1891, S. 273.

[6] Stadelmann, *Ztschr. f. Biol.*, München, Bd. xxvi. S. 491. Neumeister suggests the name tryptophan for this substance, *ibid.*, S. 324; Nencki, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxviii. S. 560.

[7] Salkowski, *ibid.*, Bd. xii. S. 648; Tappeiner, *Ztschr. f. Biol.*, München, Bd. xxii. S. 236.

[8] For recent work on the mycological processes in the intestines, see V. D. Harris, *Journ. Path. and Bacteriol.*, Edin. and London, 1895, vol. iii. p. 310. On the putrefaction of pure proteids see O. Emmerling (*Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1896, Bd. xxix. S. 2721); in addition to the substances enumerated above he finds betaine.

[9] *Journ. f. prakt. Chem.*, Leipzig, Bd. xvi. S. 129; Bd. xvii. S. 312; *Ann. d. Chem.*, Leipzig, Bd. xxxi. S. 129.

[10] Krüger, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xliii. S. 244.

compounds of this substance, but, as Liebig[1] was the first to show, this work was full of fallacies, and the only remnant of it is the survival of the word proteid.

Pavy[2] has used caustic potash in his researches on proteids, and shown that the action of the alkali is to split off a substance of an amylose nature which, on further treatment with mineral acids, yields a reducing but non-fermentable sugar, $C_6H_{12}O_6$, which gives a crystalline osazone with phenylhydrazine. Pavy, however, himself points out that he is not the first to obtain this result. Schützenberger[3] many years ago obtained from proteid a dextrin-like substance by the prolonged use of baryta water, which, after treatment with sulphuric acid, reduces Fehling's solution, and "appears to be glucose, or an analogous substance." From his own and Schützenberger's work, he draws the conclusion that proteid matter has the constitution of a glucoside. These experiments will be again referred to under the gluco-proteids.

O. Nasse[4] discovered that by boiling proteids with a strong solution of barium hydrate some of their nitrogen was disengaged as ammonia, but this only accounted for a small percentage of the total nitrogen. He concluded that the nitrogen which is readily liberated is in the form of an amide, that some is combined similarly to that of creatine, but that the major part which is unaffected by this treatment is in the form of an amido-acid.

Schützenberger[5] has elaborated the baryta method. He obtained different results by varying the conditions of temperature and pressure, of the time of treatment, and of the amount of barium hydrate employed. In his earlier researches he employed coagulated egg-white, which had been thoroughly washed with water, alcohol, and ether; weighed amounts of it were treated with from two to six times their weight of crystallised barium hydrate and with water, the whole being heated in a closed iron vessel to temperatures ranging from 100° to 250° C., and for periods of time varying from 8 to 120 hours. He found that nitrogen to the extent of about one per cent. of the total weight of albumin is given off as ammonia at atmospheric pressure, by boiling for half an hour; a second one per cent. comes off slowly by continued boiling for 120 hours (this result is, however, more easily obtained by treating with three parts of barium hydrate at 120° C. for six to eight hours); a third one per cent. is given off by treating with two parts of barium hydrate at 150° C., and a fourth one per cent. by heating with excess of barium hydrate at 260° C.

He next found that accompanying these four stages there were different cleavage products obtained. First, some insoluble barium salts, namely, oxalate, carbonate, phosphate, and sulphate. On calculating the quantities of oxalate and carbonate formed, he arrived at the interesting result that they were present in proportions to support the hypothesis that, with the ammonia set free, they had existed in the proteid molecule as a urea and oxamide radicle. The barium carbonate and oxalate, moreover, were formed at different stages of ammonia

[1] *Ann. d. Chem.*, Leipzig, Bd. lxii. S. 132.

[2] "Physiology of the Carbohydrates," London, 1894, p. 28; *Proc. Roy. Soc. London*, 1893, vol. liv. p. 53; *Rep. Brit. Ass. Adv. Sc.*, London, 1896.

[3] *Bull. Soc. chim.*, Paris, 1875, Sér. 5, tome xxiii. p. 161.

[4] *Chem. Centr.-Bl.* Leipzig, 1873, S. 137; *Arch. f. d. ges. Physiol.*, Bonn, Bde. vi. S. 589; vii. S. 139; viii. s. 381.

[5] *Ann. de chim.*, Paris, Sér. 5, tome xvi. p. 289; *Compt. rend. Acad. d. sc.*, Paris, tome ci. p. 1267; cii. p. 289; cvi. p. 1407; cxii. p. 189; *Bull. Soc. chim.*, Paris, Sér. 5, tome xxiv.

elimination, in such a way that the first amount of ammonia might be considered to come from one of the amide radicles of the oxamide, while the second corresponded to the urea, and the third to the remaining nitrogen of the oxamide, then present as oxamic acid.

After precipitating the barium with carbonic anhydride and sulphuric acid, he obtained, by distillation in a partial vacuum, a small quantity of acetic acid, traces of formic acid, and an essential volatile oil which he indentified as pyrrol contaminated with smaller quantities of methyl-pyrrol and ethyl-pyrrol. The remainder, which did not volatilise nor sublime at a low temperature, he termed *résidu fixe*. By contrasting the composition of this with that of the original albumin, and taking into account the substances already enumerated, he found that the essential action of the barium hydrate was that of hydrolysis. By repeated crystallisations from water, alcohol, and ether, he separated the constituents of his *résidu fixe* and found they were amido-acids of two classes, which we may term *A* and *B*.

A. These comprised over 80 per cent. of the total weight; in them the proportion N:O = 1:2. They consisted of—

1. Amido-acids of the series $C_nH_{2n+1}NO_2$.

These he called *leucines*. They included alanine (C = 3) in small quantities, propalanine or amidobutyric acid (C = 4), butalanine or amidovaleric acid (C = 5), both in considerable amount, and leucine or amidocaproic acid (C = 6), in very large quantities. Glycocine or amido-acetic acid (C = 2) was not found.

2. Amido-acids of the series $C_nH_{2n-1}N_2O$.

These are amido-acids of the acrylic series, and were called *leucëines*. Here, too, the term which was most abundant is that in which C = 6, but bodies corresponding to C = 4 or 5 were also found.

3. Amido-acids of the series $C_nH_{2n}N_2O_4$, or some multiple of this. To these substances he gave the name of *gluco-proteins*, on account of their sweet taste. The most abundant of these were those in which C = 9 or 7, or some multiple of these numbers; but others in which C = 8, 10, and 11 were also isolated.

B. These comprised about 16 per cent. of the total weight; in them the proportion N:O = 1:3, or 1:4, or 2:5. In this class were found—

1. Tyrosine; the amount of this was about 3·5 per cent.
2. Tyroleucine, $C_7H_{11}NO_2$, in about the same quantity.
3. Very small quantities of glutaminic acid, $C_5H_9NO_4$. This is an optically inactive amido-derivative of one of the pyrotartaric acids (glutaric).

Of these substances, Schützenberger found varying quantities, according to the degree to which the hydrolytic decomposition had been carried out. The more thorough the hydrolysation, the more leucines and leucëines were found; but in earlier stages gluco-proteins were in excess.

With other proteids he obtained corresponding results. Gelatin gave the same substances, with the addition of amido-acetic acid or glycocine; 20 to 25 per cent. of this substance was obtained.

He concluded that the albumin molecule, under the action of barium hydrate, loses ammonia, carbonic anhydride, acetic and oxalic acid, and, becoming hydrated, forms in the first instance gluco-proteins, mainly those in which C=9, or some multiple of this, and that on further action these are changed into leucines and leucëines.

2. *Treatment with acids.*—Prolonged heating of proteids with dilute acids results in their hydration and the formation of proteoses and peptone.[1] Strong acids produce the same effect at the ordinary temperature in the course of a few days.[2] This method has the disadvantage that strongly coloured materials make their appearance, and to avoid this Hlasiwetz and Habermann[3] introduced the important modification of heating with strong hydrochloric acid and stannous chloride, by which pale yellow solutions were obtained without a trace of charring. A certain amount of reduction occurred during the operations, leading to the formation of stannic chloride. These methods yielded the following substances:—

(1) Ammonia; (2) an amido-acid of the acetic series, namely, leucine; (3) two amido-acids of the acrylic series, namely, asparaginic acid ($C_4H_7NO_4$, amido-succinic acid), and glutaminic acid ($C_5H_9NO_4$, amido-pyrotartaric acid), both in considerable amount; (4) tyrosine or oxyphenylalanine.

Ritthausen[4] was the first to separate glutaminic acid and asparaginic acids from proteids. He and Kreusler suggested that glutaminic acid was a typical product of vegetable proteid; but Hlasiwetz and Habermann[5] obtained it from casein, albumin, and other animal proteids. The glutaminic acid they isolated was levorotatory, while that obtained by Schützenberger was only found in small quantities and was optically inactive. By boiling the active form with barium hydrate, however, it is converted into the inactive form, and further, the inactive form if allowed to ferment under the action of the mould *Penicillium glaucum*, is transformed into the optically active modification.[6]

Employing the same method, Horbaczewski[7] obtained leucine, glutaminic acid, and glycocine from gelatin, but no asparaginic acid or tyrosine. Hofmeister,[8] however, obtained small quantities of asparaginic acid.

From elastin were obtained ammonia, leucine, tyrosine, glycocine, butalanine ($C_5H_{11}NO_2$, amido-valeric acid), but no glutaminic or asparaginic acids.[9]

From reticulin (a substance separated from reticular tissue) were obtained ammonia, sulphuretted hydrogen, and amido-valeric acid, but no tyrosine or glutaminic acid (Siegfried).

By the employment of the same method, Drechsel[10] has discovered two new substances, which are of special interest. He began by studying the quantities of nitrogenous bodies which other observers had obtained; and if he assumed that only one-half of these had been isolated there still remained about 30 per cent. of the nitrogen of the proteid to be accounted for. He pointed out also that Schützenberger obtained carbonic anhydride by his method, and that Hlasiwetz and Habermann did not by theirs. He argued from this that there should be some other

[1] Neumeister, *Ztschr. f. Biol.*, München, Bd. xxiii. S. 381.

[2] See Brodie, *Science Progress*, London, 1895, vol. iv. p. 67.

[3] *Ann. d. Chem.*, Leipzig, 1873, Bd. clxix. S. 150. In a paper published previous to this by the same observers (*ibid.*, Bd. clix. S. 304), they sought unsuccessfully to establish a definite relationship between proteids and carbohydrates.

[4] *Journ. f. prakt. Chem.*, Leipzig, Bde. xcix. S. 454; cvii. S. 218.

[5] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1872, Bd. ix. S. 114.

[6] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xvii. S. 388. Similar changes in the optical varieties of leucine and other substances are similarly produced.

[7] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1880, Abth. 2, Bd. lxxx. S. 101.

[8] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ii. S. 299.

[9] Horbaczewski, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1886, Abth. 2, Bd. xcii. S. 657.

[10] "Der Abbau d. Eiweissstoffe," *Arch. f. Physiol.*, Leipzig, 1891, S. 248.

substance produced by the latter method, which should yield carbonic anhydride on treatment with an alkali. As he considered it probable that this substance might be basic, he endeavoured to isolate it as a precipitate by means of phosphotungstic acid, a reagent which is of great value as a precipitant of basic or alkaloidal bodies. By this means he succeeded in isolating not one but two bases, which he named *lysine* and *lysatinine*.

Lysine has the formula $C_6H_{14}N_2O_2$, and is homologous with Jaffe's[1] ornithin ($C_5H_{12}N_2O_2$, probably diamidovalerianic acid). Lysine is probably diamidocaproic acid. On heating it to 120°–130° with barium hydrate, barium carbonate is formed. It will therefore account for some of Schützenberger's carbonic anhydride.

Lysatine or *lysatinine*, the second base, is even more interesting. Its formula is either $C_6H_{13}N_3O_2$ or $C_6H_{11}N_3O$, and is a homologue of either creatine ($C_4H_9N_3O_2$) or creatinine ($C_4H_7N_3O$), according as the first or second formula is taken.

It can be obtained pure as a double salt of its nitrate with silver nitrate, and this when boiled with barium hydrate for twenty-five minutes yields urea; this is a decomposition exactly analogous to that of creatinine under the same circumstances. From 10 grms. of the double salt Drechsel obtained about 1 grm. of urea; which is a large quantity when one considers that under the conditions urea is quickly broken up into ammonia and carbonic anhydride.

This is the first time that urea has been obtained from proteid in laboratory experiments. Many years ago, Béchamp[2] stated that he had obtained urea from egg-white by the oxidising action of potassium permanganate. Lossen[3] found that the substance Béchamp took for urea was guanidine, which probably came from small quantities of xanthine, present in the egg-white as an impurity, or, as Drechsel[4] points out, from lysatinine. Drechsel's method, it is important to notice, is one of hydration; not, like Béchamp's, one of oxidation.[5]

It should be added that Drechsel's work was carried out in the first instance with casein, but his pupils have discovered lysine and lysatinine among the cleavage products of other proteids and proteid-like substances,[6] and further that the same substances are formed during pancreatic digestion.[7]

Hedin[8] has more recently arrived at the conclusion that lysatinine is not a chemical individual, but a mixture of lysine with another base called arginine. Arginine ($C_6H_{14}N_4O_2$) was originally separated from vegetable tissues by Schulze and Steiger,[9] and subsequently it was found by Hedin[10]

[1] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. x. S. 1925.

[2] *Ann. d. Chem.*, Leipzig, 1856, Bd. 100, S. 247.

[3] *Ibid.*, 1880, Bd. 201, S. 369. Between Béchamp's and Lossen's time the question was investigated by Städeler, *Journ. f. prakt. Chem.*, Leipzig, 1857, Bd. lxxii. S. 251; Loew, *ibid.*, 1871, N.F., Bd. iii. S. 289; Tappeiner, *ibid.*, 1871, Bd. civ. S. 408; and Ritter, *Compt. rend. Acad. d. sc.*, Paris, tome lxxiii. p. 1219; all of whom except Ritter failed to confirm Béchamp's results.

[4] *Loc. cit.*

[5] On the formation of urea by oxidation from many organic substances, see F. Hofmeister, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1897, Bd. xxxvii. S. 426.

[6] Fischer (from gelatin), Inaug. Diss., Leipzig, 1890; *Arch. f. Anat. u. Physiol.*, Leipzig, 1891, S. 205. Siegfried (from conglutin), *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxiv. S. 418; (from reticulin), "Ueber d. chem. Eigenschaften des retic. Gewebes," *Habilitationschrift*, Leipzig, 1892. Siegfried also obtained from conglutin a sweet substance ($C_8H_{16}N_2O_4$), corresponding to one of Schützenberger's gluco-proteins.

[7] Hedin, *loc. cit.* [8] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 297.

[9] *Ibid.*, Bd. xi. S. 43; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxiv. S. 2707; 1896, Bd. xxix. S. 352.

[10] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xx. S. 186; xxi. S. 155; xxii. S.191.

to be a constant decomposition product of proteids and albuminoids. It yields urea on treatment of its silver salt with barium hydrate.

Other recently published work on the decomposition of proteids by hydrochloric acid is by R. Cohn.[1] He used concentrated acid, and no stannous chloride. From 1000 parts of casein he recovered 916·75 of products; these consisted of fatty acids, 34·25; tyrosine, 35; leucine, 321; an oily product containing an acid, $C_7H_{18}N_3O_8$, 180; pyridine derivatives, 3·65; other substances, including cystin, cystein, thiolactic acid, and Drechsel's bases, the remainder.

3. *Treatment with oxidising agents.* — Treatment of proteids with nitric acid yields, first, an insoluble yellow substance of uncertain composition, called xanthoproteic acid; this dissolves gradually, and finally paraoxybenzoic acid (probably from the oxidation of tyrosine),[2] oxalic, fumaric, and saccharic acids are formed.

Oxidation by manganese dioxide or potassium bichromate, with sulphuric acid, has yielded—(1) Fatty acids, from formic up to caprylic ($C_8H_{16}O_2$), and their aldehydes; (2) nitrates of acetic, propionic, valeric, butyric, and hydrocyanic acids; (3) benzoic acid and benzoic aldehyde. Oxidation with chlorine water has yielded, among other products, fumaric acid, oxalic acid, and chlorazol. Oxidation with bromine water at high temperatures in a sealed tube has yielded carbonic anhydride, oxalic acid, ammonia, bromanil ($C_6Br_4O_2$), bromoform ($CHBr_3$), monobrom-benzoic acid, mono- and dibromacetic acids, tribromamidobenzoic acid ($C_6HBr_3(NH)COOH$), asparaginic and malaminic acids, leucine and leucimide ($C_6H_{11}NO$). No tyrosine was obtained.[3]

4. *Treatment by action of heat.—Dry distillation* leads to the formation of a complex oily material, called "Dippel's oil," which contains a large number of substances; among these are hydrocarbons of the fatty series, ammonium salts of fatty acids, nitrates and ketones of the same series, carbonic anhydride, ammonia, amines of fatty acid radicles, hydrocarbons and amines of the benzene series, aniline and its homologues, phenol and its homologues, members of the pyridine group of bases, namely, pyridine ($C_5H_5N_5$), picoline (C_6H_7N), lutidine (C_7H_9N), and collidine ($C_8H_{11}N$), and lastly, pyrrol.

Some of the substances last mentioned will also be found in our list of the animal alkaloids (p. 59); here we have direct proof that proteid substances have within them, or are capable of forming by intramolecular rearrangement, basic bodies of this nature. The pyridine bases have, moreover, been shown to take a part in the formation of the vegetable alkaloids, piperidine, cinchonine, etc.

It is extremely probable that proteids contain within their molecule a radicle of the closed ring series, but even if they do not, there still remains the possibility that by the action of heat, substances with open carbon chains may be transformed into those with closed rings. The following example is selected by Brodie[4]:—

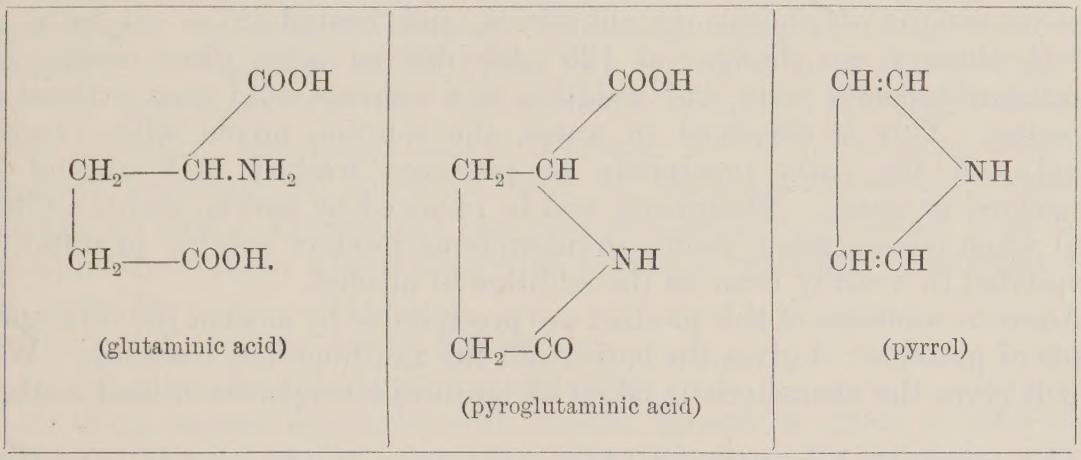
[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 153.

[2] Baumann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xii. S. 1453.

[3] Hlasiwetz and Habermann, *Ann. d. Chem.*, Leipzig, 1871, Bd. clix. S. 304. Blennard, *Compt. rend. Acad. d. sc.*, Paris, tome xc. p. 612; xcii. p. 458. This latter observer also obtained gluco-proteins.

[4] *Loc. cit.*

At 190° dry glutaminic acid is converted into pyroglutaminic acid, and at a higher temperature this, in turn, is converted into pyrrol.[1]



The attempted synthesis of proteids.—Since Wöhler in 1828 succeeded in making urea artificially from its elements, the strides that organic chemistry has made have been prodigious. Complex substances, previously made only in the living laboratory of plants and animals, are now manufactured daily in the test tubes and retorts of the chemist. The substances of most importance to vital processes, the carbohydrates and the proteids, have been among the last to yield before this advance, but we have seen how sugar has given way to Fischer; and there are signs that the last conquest of organic chemistry, the synthesis of proteids, cannot be far distant. I propose to sketch one or two of the principal attempts that have been made in the manufacture of albuminous from simpler substances.

Schützenberger's experiments.—We have already seen that the products of decomposition of a proteid are extremely numerous, but briefly they fall into two principal groups, the fatty compounds (generally containing an amidogen radicle) and the aromatic compounds or derivatives of benzene. To Schützenberger[2] belongs the credit of an attempt to build up from some of the compounds he had shown could be obtained from albumin, something like the original proteid.

In order to effect the synthesis of proteid material, he considered it necessary to combine a molecule of a leucine (*i.e.* an amido-fatty acid) with a molecule of a leucëine (an amido-acid of the acrylic series), with elimination of water, and then to combine this complex group with one or more molecules of urea and oxamide, also with the elimination of water. We have already seen that the method he had adopted for the breaking up of proteids—heating with alkali—leads to hydrolysis; so in any attempt at synthesis he recognised as a *sine qua non* the necessity of some method of dehydration.

The provisional formula he gives is the following:—

$$H_2C_2O_4+2\ NH_3+3C_mH_{2m+1}NO_2+3\ C_nH_{2n-1}NO_2$$

with elimination of eight molecules of water. This would give

$$C_{q+2}H_{2q-8}N_8O_8, \text{ and if } q=28,$$

the percentage composition calculated from the formula agrees closely with that of albumin.

[1] Bernheimer, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xv. S. 1222.
[2] *Compt. rend. Acad. d. sc.*, Paris, tome cvi. p. 1407; cxii. p. 198.

Accordingly, amido-compounds, leucines ($C_mH_{2m+1}NO_2$), and leucëines ($C_nH_{2n-1}NO_2$), were mixed with about 10 per cent. of urea, and finely powdered. The mixture was dried at 110° C., intimately mixed with 1·5 times its weight of phosphoric anhydride, and heated in an oil bath. At 120° C. there is no change; at 125° dehydration takes place rapidly, and the mixture becomes pasty, but solidifies to a compact solid mass without any darkening. This is dissolved in water, the solution mixed with excess of alcohol, and the pasty precipitate so produced washed with alcohol and re-dissolved in water. Phosphoric acid is removed by baryta, and the filtered liquid when concentrated yields an amorphous product soluble in water, but precipitated in a curdy form on the addition of alcohol.

Aqueous solutions of this product are precipitable by most of the other precipitants of proteids; it gives the biuret and the xanthoproteic reactions. When burnt it gives the characteristic odour of burning nitrogenous animal matter.

The product he obtained does not give all the proteid reactions; it is, for instance, not precipitable by acetic acid and ferrocyanide of potassium; and the evidence as to its proteid nature is otherwise hardly conclusive, because the colour tests for proteids are given by many of the decomposition products of albuminous matter. The partial success obtained will, however, point the way for future attempts, and so far as it goes, is in favour of Schützenberger's *ureide* theory of proteid constitution. Complete success could hardly have been anticipated from such an experiment, because no means were taken to ensure the presence of sulphur, an element present in all proteids. Moreover, in the synthesis, no aromatic substance was introduced; this, however, is not absolutely necessary, as the formation of aromatic from fatty compounds by heat is a familiar chemical change (see p. 34).

Grimaux's experiments.—Some years previous to Schützenberger's work, Grimaux [1] had obtained, by somewhat simpler processes, substances which even more resembled proteids in their reactions than Schützenberger's. He was engaged in studying the properties of certain substances, inorganic and organic, which he termed "*colloïdes*," and of those which he prepared the three which especially bear on the present question are the following:—

(*a*) *Colloïde amidobenzoïque.*—This is made by heating, to 125° C., meta-amidobenzoic acid in a sealed tube, with one and a half times its weight of phosphorus pentachloride, for ninety minutes. The product is a white friable powder; this is washed repeatedly with boiling water to remove all phosphoric acid. The remaining substance Grimaux supposes to be an intramolecular anhydride, formed by the union of several molecules of meta-amidobenzoic acid, with the elimination of water. When ammonia is added, it dissolves slowly in the cold, but rapidly on heating. The solution obtained is evaporated *in vacuo*, at a low temperature, and the resulting solid is a transparent jelly which dries into translucent, yellowish plates, which in their physical properties resemble dried serum albumin.

(*b*) A colloïd which is similarly prepared, except that the temperature in the sealed tube is allowed to rise to 135° C.

(*c*) *Colloïde aspartique.*—This is made by the action of a current of gaseous ammonia on solid aspartic anhydride, heated to 170° C. The

[1] *Compt. rend. Acad. d. sc.*, Paris, tome xciii. p. 771; xcvii. pp. 231, 1336, 1434, 1485, 1540, 1578; *Rev. scient.*, Paris, April 18, 1886; this article gives a summary of the other papers.

product is washed with water, and after evaporation *in vacuo* yields a substance similar in appearance to the colloid (*a*).

In all three cases, heavy molecules were formed; and in all, the result was a colloidal substance, exhibiting many of the properties of proteids. In the case of the first two colloids, there was present not only the amidogen, but also the aromatic radicle. Although the result is not albumin, the resemblance between the physical properties and chemical reactions of proteids and of these synthesised colloids is remarkably close. Pickering[1] has fully confirmed Grimaux's results, and has added new facts illustrating the points of similarity between them and proteids.

The chief of these are as follows:—

1. All give the xanthoproteic reaction.

2. With copper sulphate and caustic potash, *a* gives a blue-violet; *b*, *nil*; *c*, a typical violet coloration, like that given by albumin.

3. Their solutions do not coagulate on heating in the absence of salt; if, however, a trace of a soluble barium, strontium, or calcium salt is present, opalescence occurs at 56°, and coagulation at 75° C.

4. The colloids are removed from solution (rising to the surface of the fluid) by saturation with magnesium sulphate, sodium chloride or ammonium sulphate. Here they especially resemble the class of proteids called globulins.

5. Another resemblance to globulins is seen in their behaviour to a stream of carbonic anhydride, which, in the presence of salts, causes precipitation. The passage of a current of air through the mixture redissolves the precipitate.

6. The colloid *b* is not digested by pepsin-hydrochloric acid; *a* is slightly digested; *c* is easily digested, and the solution gives the typical peptone colour, pink, on the addition of copper sulphate and caustic potash.

7. Any one of the three colloids, when intravenously injected into animals (rabbits, cats, dogs, rats, guinea-pigs) causes extensive intravascular coagulation. In a typical experiment death is due to respiratory failure. In this property of the proteid-like colloids, which was discovered by Pickering, they closely resemble the nucleo-proteids (see p. 67). The resemblance to the action of the nucleo-proteids extends even to minor points; for instance, neither cause intravascular clotting in albino rabbits;[2] and in dogs very minute doses indeed, cause a slowing of the rate of coagulation (Wooldridge's negative phase).

The artificial colloids do not resemble nucleo-proteids in promoting the formation of fibrin in solutions of fibrinogen.

If nucleo-proteids and these colloids both produce the same effect in the same way, their physiological activity is probably connected with the presence of some radicle common to both. The colloidal condition will not explain the action, since most colloids do not act thus.

[1] Pickering, J. W., *Journ. Physiol.*, Cambridge and London, vol. xiv. p. 347; xviii. p. 54; Pickering and Halliburton, *ibid.*, vol. xviii. p. 285. More recently Pickering has succeeded in making several new proteid-like colloids (*Proc. Roy. Soc. London*, 1896, vol. lx. p. 337).

[2] This point has been worked out by Pickering also in the case of the Arctic hare. During the albino stage of the animal, neither nucleo-proteids nor synthesised colloids cause intravascular coagulation, but during their pigmented stage intravascular coagulation is produced in the usual way. The change in the external appearance of the animal is thus associated with other changes in its constitution (*Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 310).

The active radicle cannot be one which contains phosphorus, since the synthesized colloids are free from that element. It may possibly be the amido-fatty radicle in a high state of condensation.

Lilienfeld and Wolkowicz,[1] by the condensation of amido-acid compounds, have obtained substances which resemble proteoses in their reactions.

Theories of proteid constitution.—The views of Schützenberger on this subject will have been gathered from the preceding section. There now remain to be mentioned some other theories on the subject, which are in part deductions from the work of others, and partake more of the nature of speculation than of hypotheses that have been tested by experiment.

Pflüger's theory.—The distinction between non-living proteids and living protoplasm was noted as early as 1821 by Rudolphi,[2] who wrote: "The components of the dead and living body do not exist under the same chemical conditions." A few years later the distinction between living and non-living proteids was emphasised by John Fletcher.[3] Pflüger's theory[4] was, however, the first intelligible one to explain such differences. The non-living proteids, such as are contained in white of egg, are stable and indifferent to neutral oxygen; but when such proteids are assimilated—that is, become part of a living cell—the molecules live by breathing oxygen. The assimilation of a proteid is probably due to the formation of ether-like combinations between the molecules of living proteid and the isomeric molecules of the food proteid, water being eliminated; this process of polymerisation produces large and heavy but still simple molecules; and during its occurrence the nitrogen of the non-living proteid leaves the hydrogen with which it is combined in the form of amidogen (NH_2), and enters into combination with carbon to form the much more unstable substance cyanogen (CN). We thus find uric acid, creatine, guanine, etc., as products of proteid metabolism, while none of such cyanogen-containing molecules are obtainable from non-living proteid.

Pflüger's theory was put forward in 1876; but in the light of Drechsel's later work, the part involving exchange of nitrogen between cyanogen and amidogen is rendered unlikely, and with that the whole theory must probably fall.

Loew's theory.—The researches of Loew and Bokorny[5] have taken the same direction as those of Pflüger, in that they are attempts to explain the distinction between living and dead protoplasm. Living protoplasm or proteid (in the cells of various algæ) has the property of reducing silver from a weak alkaline solution of silver nitrate; dead proteid has no such effect; animal protoplasm is so quickly killed by silver nitrate, that it does not give the reaction. The conclusion formed is, that something of the nature of an aldehyde occurs in living protoplasm. Formic aldehyde is probably formed in plants by the union of carbon and water; if this is united to ammonia, aspartic aldehyde is formed, thus:—

[1] *Arch. f. Anat. u. Physiol.*, Leipzig, 1894, Physiol. Abth. S. 383 and 555.
[2] "Grundriss der Physiologie," 1821.
[3] "Rudiments of Physiology," Edinburgh, 1837.
[4] *Arch. f. d. ges. Physiol.*, Bonn, Bd. x. S. 251.
[5] "Die chem. Kraftquelle im lebenden Protoplasma," Munich, 1882. Loew's most recent views on this subject will be found in a recently published pamphlet, "The Energy of Living Protoplasm," London, 1896.

$$4CHOH+NH_3=NH_2.CH.COH+2H_2O$$
$$\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad CH_2.COH$$

By polymerisation of aspartic aldehyde we have—

$$3\left\{\begin{matrix}NH_2.CH.COH\\ |\\ CH_2COH\end{matrix}\right\}=C_{12}H_{17}N_3O_4+2H_2O$$

and by further polymerisation in the presence of a sulphur compound and hydrogen we get

$$6C_{12}H_{17}N_3O_4+H_2S+6H_2=C_{72}H_{112}N_{18}SO_{22}+2H_2O$$

which represents the composition of ordinary albumin. If such an aldehyde does exist in "living proteid" its instability is explicable, because molecular movements would be constantly occurring in the aldehyde group.

The theory is ingenious, but an obvious objection to it is that it assumes the empirical formula above given for albumin to be the correct one. The theory has been adversely criticised by Baumann [1] who points out that aldehydes are not the only substances that reduce alkaline solutions of silver nitrate, but that many organic substances, such as pyrogallol, resorcin, hydrochinon, pyrocatechuic acid, alloxan, and morphine do so also. It is stated, moreover, by Kretzschmar [2] and Griffiths,[3] that both living and dead protoplasm give the reaction.

Latham's theory.[4]—This is to some extent a combination of the two just described. Latham considers living proteid to be composed to a chain of cyanalcohols or cyanhydrins, as they are often called, united to a benzene nucleus.

A cyanalcohol is a substance obtained by the union of an aldehyde with hydrocyanic acid; for instance—

$$CH_3.COH+HCN=CH_3.CH(CN)OH$$

(ethaldehyde) (hydrocyanic acid) (cyanethylic alcohol)

Other alcohols are formed from other aldehydes, and these are all united to one another and to benzene to form a proteid.

Latham shows that the various products of the disintegration of albumin can also be obtained by the condensation and intramolecular changes that these cyanalcohols, which are exceedingly unstable substances, undergo. Instability and proneness to undergo intramolecular changes are two properties common to "living proteids" and to cyanalcohols.

General properties and reactions of proteids.—*Solubilities.*—All proteids are insoluble in alcohol and ether. Some are soluble in water, others insoluble. Many of the latter are soluble in weak saline solutions. Some are insoluble, others soluble in concentrated saline solutions. It is on these varying solubilities that proteids are classified.

All proteids are soluble with the aid of heat in concentrated mineral acids, in glacial acetic acid, and in caustic alkalis. Such treatment, how-

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxix. S. 400.
[2] *Centralbl. f. agric. Chem.*, Leipzig, 1882, p. 830.
[3] *Chem. News*, London, vol. xlviii. p. 179.
[4] *Brit. Med. Journ.*, London, 1886, vol. i. p. 629; *Lancet*, London, 1888, vol. ii. p. 751.

ever, decomposes as well as dissolves the proteid. Proteids are also soluble in gastric and pancreatic juices, but here again they undergo a change, being converted into the hydrated varieties of proteid known as proteoses and peptones. Solutions of the proteids are precipitated by a large number of reagents, but the proteoses and peptones furnish many exceptions to this statement.

The principal precipitants of proteids are:—

1. Strong mineral acids, especially nitric, metaphosphoric, and phosphotungstic acids.

2. Acetic acid with potassium ferrocyanide.

3. Acetic or oxalic acid, with excess of certain neutral salts, such as sodium sulphate, sodium chloride, or magnesium sulphate.

4. Salts of the heavy metals; basic lead acetate, mercuric chloride, silver nitrate, copper sulphate, ferric chloride or acetate, potassio-mercuric iodide, sodium tungstate, etc. The precipitates consist of the proteid in combination with variable amounts of the metal, in the form of albuminates. On the removal of the metal by a stream of sulphuretted hydrogen, the proteid is recoverable in an unchanged form.

5. Tannin; or tannin and sodium chloride together.

6. Saturation with ammonium sulphate or sodiomagnesium sulphate, or potassium acetate or carbonate. These precipitates are soluble on diluting the solution of salt in which they are suspended.

7. Picric acid.

8. Salicylsulphonic acid.

9. Trichloracetic acid.

10. Alcohol, except in the presence of free alkali, when the proteids are slightly soluble in hot alcohol.

The precipitate given by the proteoses is in many cases (as with nitric, trichloracetic, and salicylsulphonic acid, or with acetic acid and potassium ferrocyanide) soluble on heating, but re-appears when the solution cools. The greater number of the reagents mentioned do not precipitate peptone. It is precipitated completely by alcohol, tannin, and potassio-mercuric iodide, and incompletely by phosphotungstic and phosphomolybdic acids.

The following are the methods used to remove all proteid from a solution:—

1. *Brücke's method*[1] consists in the alternate addition of hydrochloric acid and potassio-mercuric iodide.

2. *Girgensohn's method*[2] consists in the addition of sodium chloride and tannin.

3. *Devoto's method.*[3]—This consists in boiling an acidulated solution of the proteid with excess of ammonium sulphate crystals; all proteids are precipitated by this means except peptone. Proteoses, if present, are precipitated but not coagulated, and can be extracted from the precipitate by water.

4. *By trichloracetic acid.*—This method consists in adding to the solution an equal volume of a 10 per cent. solution of trichloracetic acid, boiling and filtering hot. The filtrate contains the proteoses and peptone, if these are present, and the precipitate contains the other proteids. This is by far the most rapid and

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1871.
[2] *N. Repert. f. Pharm.*, München, Bd. xxii. S. 557.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xv.

simple method of separating the two classes of proteids.[1] If boiling is omitted, the proteoses are in part precipitated also (C. J. Martin).

5. *The alcohol method.*—The solution is rendered faintly acid with acetic acid, and several times its volume of absolute alcohol are added. After twenty-four hours it is boiled and filtered; the filtrate is proteid-free.[2] The action of alcohol on proteids is peculiar; it precipitates proteids in the cold; and the precipitate, if washed free from alcohol, is found to be readily soluble in suitable reagents such as saline solution. But if the precipitate is left in contact with the alcohol for days or weeks, the solubility of the precipitate is lost; the precipitate has been converted into a coagulum. This loss of solubility, however, does not occur with proteoses and peptone, and thus this is another very good though tedious method of separating native proteids from products of proteolysis.[3]

6. *Salicylsulphonic-acid method.*—This is recommended by McWilliam.[4] The reagent precipitates albumins and globulins; on heating, the precipitate is coagulated. The same reagent precipitates proteoses; on heating, the precipitate dissolves, and re-appears on cooling. The reagent does not precipitate peptones.

7. *By boiling.*—In some cases the proteids are precipitated or more properly coagulated by boiling after faintly acidulating the solution. This is the case with the albumins and globulins, and with the proteids which are usually found in morbid urines. For the separation of native proteids from proteoses and peptones, the method is not to be recommended, because boiling with even dilute acids leads to the formation of small quantities of these products of proteolysis. The use of this method has thus produced many mistakes; it led Struve, Schmidt-Mülheim, and others, to the conclusion that a peptone-like substance exists in milk and in blood; and more recently Chabrié,[5] by the use of the same method, has described a new proteose-like constituent of blood serum, to which he has given the name "albumone." Chabrié's mistake has been amply demonstrated by R. Brunner.[6] It should be added that Devoto's method is not wholly free from the same objection.[7]

For quantitative purposes the precipitate produced by these several methods may be collected, washed, dried, and weighed, then incinerated, and the ash deducted. Other methods that have been devised are densimetric methods, in which, after removal of the proteid, the loss of specific gravity is multiplied by a constant factor,[8] and methods in which, by Kjeldahl's process, a nitrogen estimation is made in the precipitate produced by some precipitant. Sebelien[9] recommends tannin for this purpose.

Precipitation by neutral salts (German, *Aussalzung*).—There are a number of organic substances which can be precipitated from their

[1] Obermayer, *Med. Jahrb.*, Wien, 1888, S. 375–381; Starling, *Journ. Physiol.*, Cambridge and London, vol. xiv. p. 131; C. J. Martin, *ibid.*, vol. xv. p. 375; Halliburton and Brodie, *ibid.*, vol. xvii. p. 169; Halliburton and Colls, *Journ. Path. and Bacteriol.*, Edin. and London, 1895, vol. iii. p. 295.

[2] Hoppe-Seyler, "Handbuch," S. 312; Schmidt, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xi. S. 10; Hoffman, *Virchow's Archiv*, 1879, November, S. 255.

[3] S. Martin, Goulstonian Lectures, *Brit. Med. Journ.*, London, 1892, vol. i.; Gourlay, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 32.

[4] *Brit. Med. Journ.*, London, 1891, vol. i. p. 837; 1892, i. p. 115. The reaction was previously described by Roch, *Pharm. Centr.-Bl.*, Leipzig, 1889, S. 549.

[5] *Compt. rend. Acad. d. sc.*, Paris, tome cxiii. p. 557.

[6] Inaug. Diss., Bern, 1894.

[7] M. Matthes, *Berl. klin. Wchnschr.*, Bd. xxxi. S. 351; Halliburton and Colls, *loc. cit.*

[8] Bornhardt, *Ztschr. f. anal. Chem.*, Wiesbaden, 1870, S. 149; 1877, S. 124; Huppert and Zahor, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 467, 484.

[9] *Ibid.*, Bd. xiii. S. 135; König and Kisch, *Ztschr. f. anal. Chem.*, Wiesbaden, Bd. xxvii. S. 191.

aqueous solutions, by the addition of certain neutral salts in large quantities; in some cases complete saturation is necessary. In some instances, as in the precipitation of urates by ammonium chloride,[1] or ammonium sulphate,[2] the formation of an insoluble compound with the base of the salt used will explain the phenomenon. In other cases, especially in the case of colloidal substances, the water-attracting power of the salt is more probably the explanation.[3] The solutions used should not be too concentrated, or the thick precipitate obtained is difficult of filtration.

The phenomenon is not confined to substances of a colloidal nature; thus, picric acid is precipitable by this means; so are soaps, especially potassium soaps by sodium chloride. But it is in connection with non-diffusible substances,[4] and especially with proteids, that the method is most used.

Proteids differ from one another a good deal in the readiness by which they are precipitated in this way. Ammonium sulphate added to saturation, precipitates all proteids except peptones[5] and certain forms of deuteroalbumose.[6] Half saturation with the same salt is sufficient to precipitate globulins,[7] acid and alkali albumin and caseinogen. Speaking generally, the globulins and nucleo-proteids are more readily precipitable by neutral salts than the albumins. Thus, globulins are precipitated by magnesium sulphate and sodium chloride, whereas albumins are not, and some globulins, like fibrinogen, are precipitated by half-saturation with sodium chloride. If the operations are carried out at the temperature of the air, the precipitated proteids are not coagulated, but are soluble in suitable liquids; and they then again show their characteristic properties.[8]

Heat coagulation.—The albumins, globulins, and some nucleo-proteids are coagulated at different temperatures, by heating their solutions. The temperature varies with the reaction of the solution,[9] the quantity and nature of the salts present[10] (minute quantities of calcium salts favour heat coagulation as they do ferment coagulation),[11] and under certain circumstances, especially in an alkaline solution, with its concentration.[12]

[1] F. G. Hopkins, *Journ. Path. and Bacteriol.*, Edinburgh and London, 1893, vol. i. p. 451.

[2] A. Edmunds, *Journ. Physiol.*, Cambridge and London, 1894–5, vol. xvii. p. 451.

[3] O. Nasse, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xli. S. 504; F. Hofmeister and S. Lewith, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1888, Bd. xx. S. 247; xxv. S. 1.

[4] On the precipitation of colloid carbohydrates by salts, see Pohl, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 151; R. A. Young, "Proc. Physiol. Soc.," 1896–97, p. xvi. in *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi.

[5] Wenz, *Ztschr. f. Biol.*, München, Bd. xxii. S. 1.

[6] Kühne, *ibid.*, Bd. xxiv. S. 1 and 308; Chittenden, *Journ. Physiol.*, Cambridge and London, vol. xvii. p. 48.

[7] Kauder, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xx. S. 411.

[8] On the precipitation of proteids by numerous salts, see Denis, "Mémoire sur le sang," p. 39; Schäfer, *Journ. Physiol.*, Cambridge and London, vol. iii. p. 181; Halliburton, *ibid.*, vol. v. p. 177; vii. p. 321; Hammarsten, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 424.

[9] Traces of acid lower, of alkali raise, the temperature of coagulation; more than traces convert the proteid into acid or alkali-albumin respectively, and these substances do not coagulate by heat.—Halliburton, *Journ. Physiol.*, Cambridge and London, vol. v. p. 165.

[10] Limbourg, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 450.

[11] Ringer and Sainsbury, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 170.

[12] Haycraft, *Brit. Med. Journ.*, London, 1890, vol. i. p. 167.

The temperature of heat coagulation of some of the principal proteids may be approximately stated as follows:—

Albumins.		Globulins.	
Egg albumin	73° C.	Cell globulin	48°–50° C.
Serum albumin (α)	73° „	Fibrinogen	56° „
„ (β)	77° „	Serum globulin	75° „
„ (γ)	84° „	Myosinogen	56° „
Muscle albumin	73° „	Vitellin	75° „
Lact-albumin	77° „	Crystallin	73° „
		Hæmocyanin	68° „

With regard to the separation of proteids by the use of the method of fractional heat-coagulation, the opinion has been expressed by Haycraft that the results obtained are not trustworthy. It is probable, nevertheless, that the method is trustworthy, since the proteids so separated can be shown to possess other differences.[1]

Mechanical precipitation of proteids.—By mechanical means, such as shaking with sand, or even pouring from one test tube to another, a solution of egg-white deposits threads of insoluble proteid, reminding one of fibrin filaments, which also they resemble in their difficulty of solubility. By prolonged shaking, 96 per cent. of the proteid present may be deposited. Other proteids behave similarly, but as a rule less markedly, namely, egg globulin, vitellin, the proteids of blood plasma, myosinogen, potato proteid, plant vitellin, alkali albumin, and some specimens of caseinogen (Ramsden).[2]

Indiffusibility.—The proteids belong to the class of substances called colloids by Thomas Graham; that is, they pass with difficulty or not at all through animal membranes, or vegetable parchment, the substance usually employed in the construction of dialysers. Proteids may thus be separated from diffusible (crystalloid) substances, like sugar and salts. If a mixture of albumin and globulin, dissolved in a saline medium as in blood serum, is placed in a dialyser, with distilled water outside, the salts and extractives pass through the membrane into the water, and water passes in; the proteids remain within; the albumin in solution, but the globulin, which is insoluble in water containing no salts, precipitated.

The term colloid does not necessarily imply that the indiffusible substances are not capable of crystallisation; for many of the proteids have now been crystallised; this is particularly the case with the vegetable proteids (p. 52), with hæmoglobin (p. 61), with egg albumin, and with serum albumin. F. Hofmeister[3] was the first to crystallise egg albumin; a solution of egg white is mixed with an equal volume of saturated solution of ammonium sulphate, and the globulin so

[1] The following are the principal papers on this question:—Halliburton on "Proteids of Serum," *Journ. Physiol.*, Cambridge and London, vol. v. p. 159; xi. 456; Corin and Berard, "Egg White," *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, 1888, tome xv. p. 4; Colin and Ansiaux, *ibid.*, 1891, tome xxi. p. 3; Haycraft and Duggan, *Brit. Med. Journ.*, London, 1890, vol. i. p. 167; *Proc. Roy. Soc. Edin.*, 1889, p. 351; *Centralbl. f. Physiol.*, Leipzig, Bd. iv. S. 1; Fredericq, *ibid.*, Bd. iii. S. 601; Chittenden and Osborne on "Corn-Proteids," *Am. Chem. Journ.*, Baltimore, vol. xiii. pp. 7 and 8; xiv. p. 1; Hewlett, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 512; Ramsden, *Proc. Physiol. Soc.*, London, 1892, p. 23; A. di Frassineto, *Sperimentale*, Firenze, 1895, tome xlix. All the above except Haycraft and Ramsden defend the method.

[2] *Arch. f. Physiol.*, Leipzig, 1894, S. 517.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 165; 1892, xvi. S. 187; see also Gabriel, *ibid.*, 1891, Bd. xv. S. 456.

precipitated is filtered off. The filtrate is allowed to stand at the temperature of the air, and as it gets concentrated minute spheroidal globules of varying size, and finally minute needles, either aggregated or separate, make their appearance (Fig. 9). On examining these crystals, they are found to consist of egg albumin, with a variable (but usually small) admixture of ammonium sulphate. Serum albumin has similarly been obtained by Gürber and Michel,[1] in a crystalline form, from the blood serum of horses and rabbits. More recently still, caseinogen has been crystallised. When a solution of this substance is mixed with

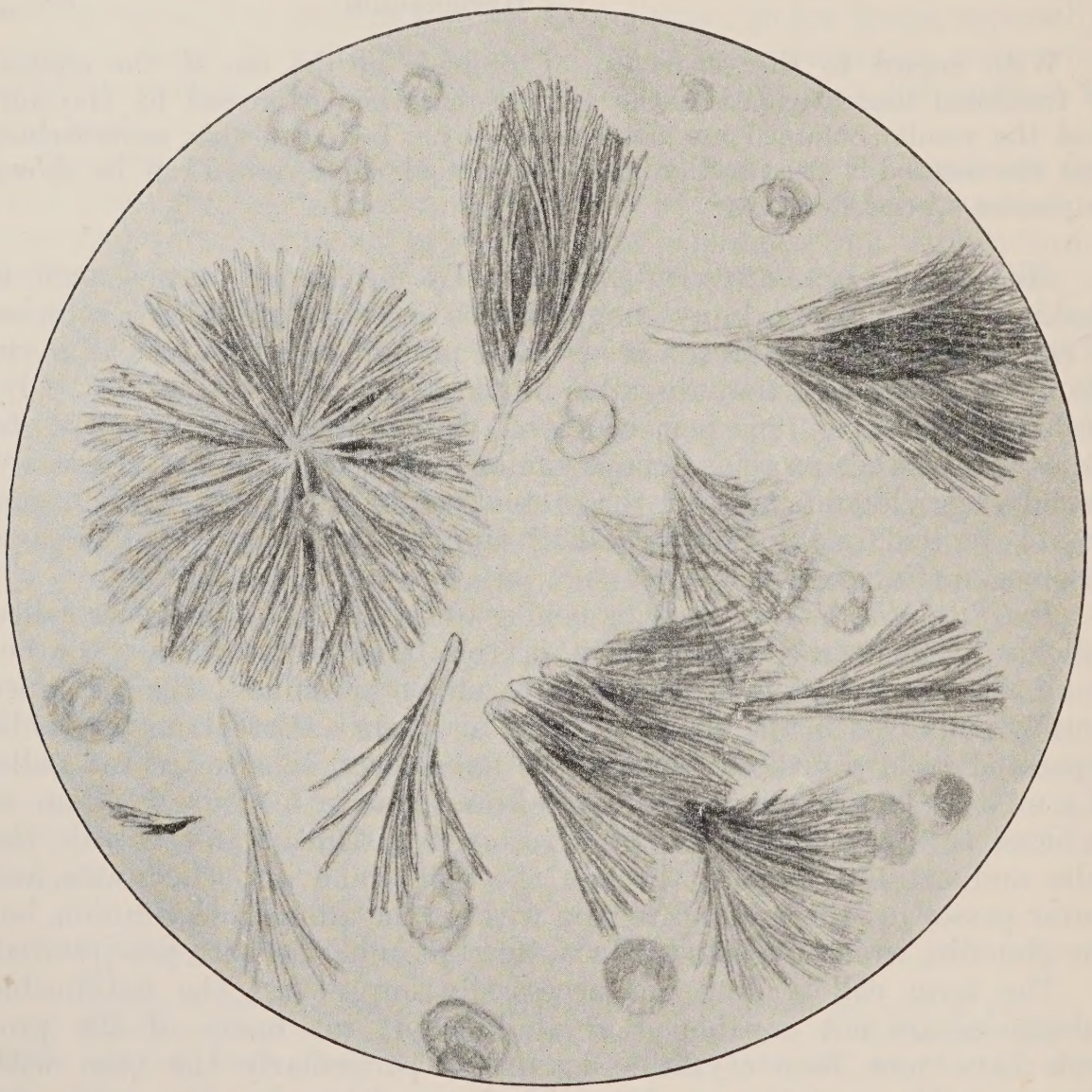


FIG 9.—Crystals of egg albumin.

ammoniacal magnesia mixture, it proceeds after some days to deposit sphæroliths, and ultimately aggregations of needle-like crystals. They contain 45 per cent. of ash, and 14·98 per cent. of nitrogen. Nuclein also yields a crystalline deposit with ammoniacal magnesia mixture (v. Moraczewski).[2]

Byrom Bramwell and Noël Paton[3] have described a case of album-

[1] *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1894. Michel (*ibid.*, No. 3, Bd. xxix.; *Centralbl. f. d. med. Wissensch.*, Berlin, 1896, S. 152) gives full details of the method employed. The crystals are hexagonal prisms with the following percentage composition:—C, 53·1; H. 7·1; N, 15·9; S, 1·9; O, 22·0; ash, only 0·22. They coagulate at 51° – 53° C. $(\alpha)_D = -61°$.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxi. S. 71.

[3] *Rep. Lab. Roy. Coll. Phys.*, Edinburgh, 1892, vol. iv. p. 47.

inuria, in which the urine on standing deposited the proteid matter in a crystalline form (see Fig. 10). They considered it to be of the nature of a globulin. Huppert[1] has questioned this conclusion, and thinks it probable that the proteid was heteroalbumose.

It is not, therefore, upon the non-crystalline character of proteid, but upon the enormous size of the proteid molecules, whether crystalline or non-crystalline, that the difficulty of diffusion depends. It thus becomes interesting to inquire into the diffusibility of the proteids of lower molecular weight, namely, the proteoses and peptones. Peptones are diffusible; this has long been known; they are highly diffusible compared to albumin, but of low diffusibility as compared with salt.

FIG. 10.—Proteid crystals from human urine.—After Byrom Bramwell and Noël Paton.

The diffusibility of the proteoses has long been inferred, but it is only quite recently that it has been accurately made out that they are intermediate in this character between peptones and albumins. The work in this direction was done independently by Kühne[2] and Chittenden,[3] and both arrived at the same results. A curious fact found was, that deuteroproteose (generally regarded as intermediate between the other proteoses and peptones) is less diffusible than protoproteose. But this

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 500.
[2] *Ztschr. f. Biol.*, München, Bd. xxix. S. 1.
[3] *Journ. Physiol.*, Cambridge and London, vol. xiv. p. 483.

is quite in accordance with Sabanejeff's [1] cryoscopic determination of the molecular weights of these substances; he gives the molecular weight of protoproteose as 2467 to 2640, of deuteroproteose as 3200, and of peptone as 400 or less. The diffusion power of the different substances investigated by Kühne was as follows:—Heteroproteose is the least diffusible of the proteoses; in neutral saline solutions it is precipitated as the salt passes out, and none goes through the dialyser; dissolved in ammonia it loses 5·22 per cent. Deuteroproteose comes next (loss, 24·1 per cent.); then protoproteose (loss, 28·3 per cent.); while peptone loses 51 to 51·8 per cent. Each experiment lasted twenty-four hours.

Action on polarised light.—All the proteids are levorotatory. The specific rotatory power of some of the principal members of the group is as follows:—

Proteids.	Observer.	Value of $(\alpha)_D$.
Serum albumin	Hoppe-Seyler [2]	−56°
	Starke [3]	−68°
Egg albumin	Hoppe-Seyler	−35°·5
	Haas [4], Starke	−38°·08
Lact-albumin	Sebelien [5]	−36°–37°
Serum globulin	Haas	−59°·75
Fibrinogen	Herrman [6]	−43°
Alkali albumin	Haas	−62°·2
Syntonin (prepared from myosin)	Hoppe-Seyler	−72°
Casein (dissolved in $MgSO_4$ solution)	Hoppe-Seyler	−80°
Various proteoses	Kühne and Chittenden [7]	−70°–80°

Colour reactions.—These are numerous, and doubtless depend for their occurrence on the various radicles which, as we have seen, are probably present in the proteid molecules. Many of them are given by certain of the decomposition products of the proteids; and by a careful comparison of these simpler substances, conclusions have been reached concerning the particular groups in the proteid molecule to which each reaction is due.

The majority of the colour tests are due to the presence of the aromatic radicle; it will, therefore, be well to preface the description of the reactions themselves by a classification of the aromatic substances derived from proteids by putrefaction. Salkowski [8] arranges them into three groups; whether all these groups exist pre-formed in the proteid molecule, or are derived, as Maly considered, from only one aromatic group, matters but little in the question under investigation. The groups are as follows:—

First group—The phenol group.—This includes tyrosine, the aromatic hydroxy acids, phenol, and cresol.

Second group—The phenyl group.—This includes phenylacetic and phenylpropionic acids.

[1] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxvi. S. 385.
[2] *Ztschr. f. Chem.*, Leipzig, 1864, S. 737.
[3] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xi. S. 17.
[4] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xii. S. 378; *Chem. Centr.-Bl.*, Leipzig, 1876, S. 295, 811, 824.
[5] *Jahresb. ü. d. Förtschr. d. Thier-Chem.*, Wiesbaden, Bd. xv. S. 184.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xi. S. 508.
[7] *Ztschr. f. Biol.*, München, Bd. xx. S. 51.
[8] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 215.

Third group—The indol group, of which indol, skatol, and skatol-carbonic acid are the most important members.

We can now proceed to the consideration of the proteid colour reactions.

1. *The xanthoproteic reaction.*—This is characterised by the yellow colour given by boiling with nitric acid, turned orange by ammonia. O. Loew[1] considered that the yellow material was a mixture of oxynitro-, trinitro-, and hexanitro-albumin; but these substances are very doubtful as chemical individuals. Salkowski found the reaction to be given by all the members of his first and third groups of aromatic substances. Pickering[2] found that salicylic acid, and salicylsulphonic acid, cholesterin, cholalic acid, and taurocholic acid also give the test. A large number of other organic substances which were tested did not give the same result. It was noticed that bodies with a benzene nucleus with one hydrogen replaced by hydroxyl, give the xanthoproteic reaction, whereas substances which contain a benzene nucleus without the hydroxyl, as phenylacetic and benzoic acids, do not.

Millon's reaction.—A brick-red coloration occurs when proteid matter is boiled with Millon's reagent (a mixture of the nitrates of mercury with excess of nitric acid); the reaction was thought by Kühne[3] to be due to tyrosine. Salkowski also took this view, as the reaction is given by the substances in his first group, the most prominent member of which is tyrosine. Those in the second and third groups do not give the test. Nasse,[4] however, demonstrated that Millon's reaction is due to benzene derivatives, in which one hydrogen atom has been replaced by hydroxyl (hydroxybenzene nucleus) and not to tyrosine. That Nasse's view is correct is shown by the following considerations:—Kühne and Chittenden[5] have found that certain anti-products of digestion, which yield neither leucine nor tyrosine on further digestion, or on decomposition with sulphuric acid, do *not* give the reaction. Schützenberger[6] found that tyrosine is absent from the putrefaction products of gelatin. Now, Salkowski stated that gelatin does not react with Millon's reagent. But Chittenden and Solley[7] have found that the products of gelatin digestion give a characteristic reaction, and Pickering that pure gelatin and gelatinoses give it in a marked manner; thus confirming the statement made by Millon[8] in his original memoir. Gelatin, therefore, owes this property to something which is not tyrosine, but which, like tyrosine, contains a hydroxybenzene nucleus.

Adamkiewicz' reaction.[9]—If glacial acetic acid in excess and then concentrated sulphuric acid are added to proteid, a violet colour with feeble fluorescence is produced. The test is by no means a certain one, and is given by proteoses and peptones in concentrated solutions only. It is not given by gelatin (Hammarsten).

This test is only given by the aromatic substances of Salkowski's third (indol) group. The strong reagents added are likely to produce

[1] *Journ. f. prakt. Chem.*, Leipzig, N.F., Bd. iii. S. 180.
[2] *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 372.
[3] *Ztschr. f. d. ges. Naturw.*, Halle, Bd. xxix. S. 506; *Virchow's Archiv*, Bd. xxxix. S. 130.
[4] *Chem. Centr.-Bl.*, Leipzig, 1879, Bd. x.
[5] *Ztschr. f. Biol.*, München, Bd. xxii. S. 423.
[6] Article in Wurtz' "Dict. de chim.," 1886, Suppl. 1 A, p. 58.
[7] *Journ. Physiol.*, Cambridge and London, vol. xii. p. 23.
[8] *Compt. rend. Acad. d. sc.*, Paris, tome xxviii. p. 40.
[9] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. viii. S. 761. See also Wurster, *Chem. Ztg.*, Cöthen, Bd. xi. S. 187.

considerable change in the proteid molecule; indol and skatol can hardly be considered to be simple cleavage products of the proteid molecule (see p. 29).

Liebermann's test[1] is performed by precipitating the proteid by alcohol, and then heating the washed precipitate with strong hydrochloric acid. The result is a violet-blue colour. The reaction is not given by pure peptone.[2] It is also not given by any of the aromatic putrefactive products of proteid, nor by a large number of other cleavage products of proteid which Pickering worked with. Its cause is therefore at present unknown.

Krasser's reaction.[3]—Alloxan in solution stains proteid matter a brilliant red. It reacts in the same way with asparagin, aspartic acid, and tyrosine. The reaction is probably connected with the presence of amido groups.

Piotrowski's reaction.[4]—If a few drops of dilute copper sulphate solution are added, and then excess of strong solution of caustic soda and potash, a violet solution is the result. If ammonia is used instead, a blue solution is formed.

In the case of the proteoses and peptones, the result is a rose-red solution with potash[5] and a reddish violet with ammonia. As the same colour is given by the decomposition product of urea called biuret,[6] the test is often called the *biuret reaction* ($2CON_2H_4 - NH_3 = C_2O_2N_3H_5$). Biuret yields, on decomposition, compounds which contain cyanogen; for instance, by heat it is split into ammonia and cyanuric acid, $(CN)_3H_3O_3$. Biuret, cyanuric acid, xanthine, hypoxanthine, sarcosine, hydrocyanic acid, all give similar reactions to the proteids. Gnezda[7] considered it probable that the biuret reaction was due to a cyanogen radicle, and that the cyanogen in albumin and peptone is differently combined, corresponding to the similar differences in cyanuric and hydrocyanic acid respectively. Pickering,[8] however, concludes, that the radicle in question is not CN but CONH.

The related metals, nickel (Gnezda) and cobalt (Pickering) give corresponding colour reactions, which may be summarised in the following table:—

Proteid.	Copper Sulphate and Ammonia.	Copper Sulphate and Potash.	Nickel Sulphate and Ammonia.	Nickel Sulphate and Potash.	Cobalt Sulphate and Ammonia.	Cobalt Sulphate and Potash.
Native proteids (albumins, globulins, and nucleo-proteids)	Blue	Violet	Nil	Yellow	Nil	Heliotrope-purple
Products of proteolysis (proteoses and peptones)	Violet	Rose-red	Yellow	Orange	Nil	Red-brown

Pickering found that when a cobalt salt has entered into the proteid

[1] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xvii. S. 8; *Chem. Centr.-Bl.*, Leipzig, 1887, Nos. 18 and 25.
[2] Le Nobel, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xvii. S. 3.
[3] *Monatsh. d. Chem.*, Wien, Bd. vii. S. 673.
[4] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. xxiv. S. 335.
[5] Brücke, *Monatsh. d. Chem.*, Wien, Bd. iv.
[6] Wiedemann, *Ann. d. Phys. u. Chem.*, Leipzig, Bd. lxxiv. S. 67.
[7] *Proc. Roy. Soc. London*, 1889, vol. xlvii. p. 202.
[8] *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 347.

molecule, it can be easily displaced by a nickel salt, and then in turn by a copper salt, each yielding its characteristic colour reaction. He examined these and other reactions in connection with various albuminoids as well; the addition of cobalt sulphate and potash to gelatin he found to produce a play of colours in the order of those of the spectrum, commencing with violet.

Drechsel[1] has drawn attention to an old observation of Krukenberg's, that at the boiling temperature there is in the so-called biuret reaction a reduction of the cupric to cuprous oxide; the latter, however, remains in solution. Drechsel shows that the reduction also occurs at the ordinary temperature. C. J. Martin[2] is also of opinion that the biuret reaction is a reduction. He finds that alkali albumin dissolves cuprous oxide and forms a pink solution, never violet or purple; these latter colours, when the test is ordinarily performed with copper sulphate, are due to admixture with cupric hydrate, held in solution by the proteid and not reduced. The most powerful reducing proteids are proteoses and peptone, hence the pink biuret reaction; whereas the native proteids have a smaller reducing power, and the pink colour is mixed with the blue cupric hydrate, and so the colour obtained is a violet.

From the preceding study of the properties and reactions of the proteids, it will be gathered that since many other substances give the same tests, a proteid can only be identified by employing a large number of its reactions. Winternitz[3] recommends a combination of a precipitant and colour reactions. The precipitant he has chiefly used in cases of albuminuria is acetic acid and potassium ferrocyanide. The precipitate so obtained gives the colour reactions well. This is also the case with the precipitate produced by several other reagents, among which may be mentioned salicylsulphonic acid,[4] and the halogens.[5]

Classification of Proteids.

It will be seen from the preceding description of the proteids, that I have used the term proteids throughout as an equivalent for albuminous substances (German, *Eiweisskörper*); certain other substances (such as hæmoglobin, mucin, nucleo-proteids) named proteids, by Hammarsten, Neumeister, and other continental writers, will be treated separately as compound proteids.

The proteids may be divided into those of animal and those of vegetable origin. There does not appear to be any essential difference between these two classes, and each can be subdivided in the same manner into sub-groups, but the distinction is a convenient one in practice.

Animal proteids.—Class 1. *Albumins.*—These are proteids which are soluble in water, in dilute saline solutions, and in saturated solutions of sodium chloride and magnesium sulphate. They are, however, precipitated by saturating their solutions with ammonium sulphate. Their solutions are coagulated by heat, usually at 70°–73° C. Serum albumin, egg albumin, lact-albumin are examples.

Class 2. *Globulins.*—These are proteids which are insoluble in water, soluble in dilute saline solutions, and insoluble in saturated solutions of sodium chloride, magnesium sulphate, and in half-saturated solution of

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xxi. S. 68.
[2] Private communication to author.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xv. S. 187; xvi. S. 439.
[4] Pickering, *loc. cit.*, p. 377.
[5] F. G. Hopkins, *Proc. Physiol. Soc.*, June 12, 1897.

ammonium sulphate. Their solutions are coagulated by heat, the temperature of heat coagulation varying considerably. Fibrinogen, serum globulin, globin, paramyosinogen, and myosinogen, crystallin, vitellin,[1] egg globulin are examples.

The differences in solubility of these two important classes of native proteids may be stated in tabular form as follows:—

Reagent.	Albumin.	Globulin.
Water	Soluble	Insoluble
Dilute saline solution	,,	Soluble
Saturated solution of magnesium sulphate or sodium chloride	,,	Insoluble
Half-saturated solution of ammonium sulphate	,,	,,
Saturated solution of ammonium sulphate .	Insoluble	,,

Class 3. *Albuminates.*—These are proteids derived from either albumins or globulins by the action of weak acids or alkalis. The term has been extended to include metallic compounds of the proteids, but restricting it here to acid albumin or syntonin, and alkali albumin, the class may be defined as consisting of proteids which are insoluble in water, and in neutral solutions containing no salt. They are soluble in acid or alkaline solutions, and in weak saline solutions. They are precipitated by neutralisation, and resemble globulins in their behaviour to neutral salts. Their solutions are not coagulated by heat.

A less soluble variety of these proteids, called Lieberkühn's jelly, is formed by adding strong acid or alkali respectively to undiluted white of egg.

Caseinogen, formerly regarded as a member of this group, will be studied with nucleo-proteids and with milk.

After egg albumin is treated with formaldehyde it remains soluble in water, but is not coagulable on heating.[2]

Class 4. *Products of proteolysis; proteoses and peptones.*—These will be studied in detail in connection with digestion. They can, however, be formed by other hydrolysing agencies than digestive juices, such as treatment with mineral acids, or superheated steam.[3] The term proteose for the intermediate products of hydration is a convenient general name, which includes not only albumoses, but also vitelloses, globuloses, caseoses, myosinoses, and the like.

Class 5. *Coagulated proteids.*—This class includes the proteids in which coagulation has been produced by heat, and those in which coagulation has been induced by ferment action, such as fibrin, myosin, casein, and anti-albumid, an insoluble by-product formed in gastric digestion.

Since the individual members of these groups have either been described in preceding sections, or will be discussed elsewhere under other heads, such as blood, milk, etc., they need not be further considered in this place.

[1] Vitellin, unlike other globulins, is not precipitated by sodium chloride. Some regard it as a nucleo-proteid. It will be more fully discussed later.

[2] Blum, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 127; *Berl. klin. Wchnschr.*, 1897, Bd. xxxiii. S. 1043.

[3] On "Atmid-albumoses" (that is, those formed by superheated steam) see Neumeister, *Ztschr. f. Biol.*, München, Bd. xxvi. S. 57; Chittenden and Meara, *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 501.

Vegetable proteids.—The amount of proteid matter in plants, especially in those which are full grown, is less than in animals. It occurs dissolved in their juices, or in their protoplasm, or deposited in the form of granules called aleuron grains. Plant proteids have frequently been obtained in a crystalline form. They may be divided into the same classes as the animal proteids.

Class 1. *Albumins.*—Small quantities of true albumin have been described by S. Martin [1] in the juice of the papaw fruit, and by Green [2] in the latex of several caoutchouc-yielding plants of the natural orders Apocyneæ and Sapotaceæ.

Class 2. *Globulins.*—These are by far the most abundant natural proteids present in plants. This view, which was taken by Hoppe-Seyler,[3] is contrary to that of Ritthausen,[4] who regarded vegetable proteids as consisting chiefly of legumin and allied substances.[5]

Class 3. *Albuminates.*—Acid and alkali albumin are formed readily from vegetable proteids, especially from plant myosin. Legumin or vegetable casein was used synonymously with vegetable proteid by some of the earlier investigators,[6] but it is now usually regarded as alkali albumin, formed artificially in the extraction of the globulins by alkali. The name conglutin was introduced by Ritthausen [7] for the more glutinous product obtained from almonds and lupins.

Class 4. *Proteoses and peptones.*—Proteoses have been described in latex, in papaw juice, and flours of different kinds. True peptones are not found in the circulating juices of plants. Probably the circulating proteids in plant life are proteoses, hemialbumoses (Vines), though amido-acids (leucine, tyrosine, asparagine, adenine, etc.) [8] also occur. These substances are formed by proteolytic ferments during germination. The best known of these ferments, papain, has been investigated by Wurtz, Martin, and others. Such ferments, as well as malting ferments, which convert the insoluble starch of the seed into the soluble sugar, are probably almost ubiquitous.[9] In carnivorous plants, another ferment is met with of a somewhat different character.

Class 5. *Coagulated proteids.*—Vegetable albumin and globulin, like those of animal origin, are converted at a high temperature into an insoluble heat coagulum.

With regard to the value of vegetable proteids as food, it may be stated that as a rule they are not nearly so readily digested as animal proteids. Prausnitz [10]

[1] *Journ. Physiol.*, Cambridge and London, vol. vi. p. 336.

[2] *Proc. Roy. Soc. London*, vol. xl. p. 28.

[3] "Physiol. Chem.," S. 75.

[4] *Ztschr. f. Chem.*, Leipzig, Ser. 2, Bd. iv. S. 528, 541; vi. 126; *Journ. f. prakt. Chem.*, Leipzig, Bd. ciii. S. 65, 78, 193, 273.

[5] Ritthausen defends his view in *Chem. Centr.-Bl.*, Leipzig, 1877, S. 567, 578.

[6] Einhof, *Neue allg. Journ. d. Chem.*, v. A. Gehlen, 1805, Bd. vi. S. 126, 548. Dumas and Cahours, Liebig, and others also examined this substance.

[7] *Ibid.*, Ser. 2, Bd. xxvi. S. 440.

[8] E. Schulze and E. Kisser, *Landw. Versuchs Stat.*, Berlin, Bd. xxxvi. S. 1; E. Schulze, numerous papers in *Ztschr. f. physiol. Chem.*, Strassburg. See especially Bd. xii. S. 405.

[9] Gorup-Besanez, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1874, S. 1478; Krauch, *Journ. Chem. Soc.*, London, 1878, Abst. p. 996; Green, *Proc. Roy. Soc. London*, vol. xli. p. 466; Thiselton Dyer's Presidential Address, Sect. D, Brit. Assoc., 1888; Hansen, *Bot. Ztg.*, 1886, S. 137; Ellenberger and Hofmeister, *Centralbl. f. agric. Chem.*, Leipzig, 1888, S. 319. The subject of enzymes and reserve materials in plants, however, is now a very large one, and it will be found discussed, with bibliography, in a series of articles by J. Reynolds Green, in *Science Progress*, London, vol. i. p. 342; ii. p. 109; iii. pp. 68, 376; v. p. 60.

[10] *Ztschr. f. Biol.*, München, Bd. xxiv. S. 227.

experimented with beans; he found the fæces contained as much as 30·3 of the nitrogen of the beans in an undigested condition. Beans thus compare unfavourably with lentils and bread, but even here there is a considerable undigested residue. The investigations of Rutgers [1] point to the fact that this is due rather to the admixture of vegetable proteids with cellulose and other indigestible materials than to any peculiarity in the proteids themselves.

The foregoing brief account of the vegetable proteids may be amplified by further consideration of some of the points raised :—

Researches on crystallised vegetable proteids.—In 1855, Hartig [2] pointed out the existence of crystallised proteid matter in seeds. Four years later, Maschke [3] obtained hexagonal plates of proteid matter by extracting Brazil nuts with water at 40°–50° C., and evaporating the filtered extract at 40°. Nägeli [4] designated such crystals as crystalloids. Weyl [5] identified the crystals as vitellin. Sachsse,[6] by Maschke's method, and also by precipitating the aqueous extract by a stream of carbonic anhydride, obtained several preparations of proteid from Brazil nut which he analysed. The precipitate consisted of small discs, not crystals. Schmiedeberg [7] obtained crystalline products from the carbonic anhydride precipitate by digesting it with magnesia solution at 35° C., and evaporating at the same temperature. Drechsel [8] obtained hexagonal crystals, by submitting the solution containing Schmiedeberg's magnesia compound to dialysis against alcohol, and also by the slow evaporation of a warm sodium chloride solution of the proteid.[9] At Drechsel's suggestion, Grübler [10] applied this method with some modifications to the proteids of squash seed, from which he obtained octahedral crystals; he obtained lime as well as magnesia crystalline compounds. Ritthausen,[11] by similar methods, obtained octahedra and rhombic dodecahedra from expressed hemp cake, castor-oil seeds, and seeds of *Sesamum indicum*. Molisch [12] has separated by the use of ammonium sulphate a crystalline proteid (phycocyanin) from the alga, *Oscillaria leptotricha*. Vines [13] found that the natural crystalloids, embedded in the ground substance of the aleuron grains, were hexagonal rhombohedra in some plants, and regular tetrahedra in others.

Some of the details of Vines' work are as follows :—

The aleuron grains of the peony contain an albumose and vegetable myosin; of the castor-oil plant, an albumose, a myosin, and vitellin; of blue lupin, chiefly crystalloid vitellin. He classified aleuron grains into—

1. Those soluble in water, albumose.
2. Those soluble in 10 per cent. sodium chloride solution—

(*a*) Without crystalloids, soluble in saturated sodium chloride solution, vitellin.

(*b*) With crystalloids, insoluble in saturated sodium chloride solution, myosin.

3. Those partially soluble in 10 per cent. sodium chloride solution. Some of these are crystalloid, some insoluble, some soluble in saturated salt solution.

Vitellin is the principal constituent of egg yolk, and occurs there in the form of semicrystalline sphærules, corresponding to the crystalloid aleuron grains of plants. The proteids described by Valenciennes and Fremy [14] in the

[1] *Ztschr. f. Biol.*, München, Bd. xxiv. S. 251. [2] *Bot. Ztg.*, 1855, S. 881.
[3] *Journ. f. prakt. Chem.*, Leipzig, Bd. lxxiv. S. 436.
[4] *Bot. Mitth.*, München, 1863, Bd. i.
[5] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xii. S. 635; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. i. S. 72.
[6] "Die Farbstoffe, Kohlenhydrate und Proteinsubstanz," Leipzig, 1877, S. 315.
[7] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. i. S. 205.
[8] *Journ. f. prakt. Chem.*, Leipzig, Bd. xix. S. 331.
[9] See Grübler, *ibid.*, Bd. xxiii. S. 100. [10] *Ibid.*, Bd. xxiii. S. 97.
[11] *Ibid.*, Bd. xxiii. S. 481. [12] *Bot. Ztg.*, 1895, Bd. i. S. 131.
[13] *Proc. Roy. Soc. London*, vol. xxviii. p. 218; xxx. p. 387; xxxi. p. 62.
[14] *Ann. de chim.*, Paris, Sér. 3, tome l. p. 129; *Ann. d. Chem.*, Leipzig, Bd. cxxvii. S. 188.

yolks of fishes' eggs, and termed by them ichthin, ichthulin, and emydin, are regarded by Hoppe-Seyler as doubtful chemical units, and are probably mixtures of vitellin with nuclein and lecithin. Whether vitellin contains phosphorus in its molecule or not is a moot point. Some regard it as a nucleo-proteid rather than a globulin; others look upon the phosphorus generally found in it as belonging to either nuclein or lecithin, adherent to it as an impurity. The same question arises in connection with phytovitellin (vegetable vitellin). Recent analyses by Osborne[1] show that it contains no phosphorus, though Sachsse, one of the earlier workers, described the presence of this element.

Proteids of flours.—Sidney Martin[2] found the principal proteids in wheat flour to be (1) a vegetable myosin, and (2) a soluble proteose, which he called phytalbumose.

Gluten is a mixture of two substances—

(*a*) Gluten fibrin, which is insoluble in alcohol, and formed from the myosin; and

(*b*) A proteose insoluble in water, formed from the phytalbumose. This

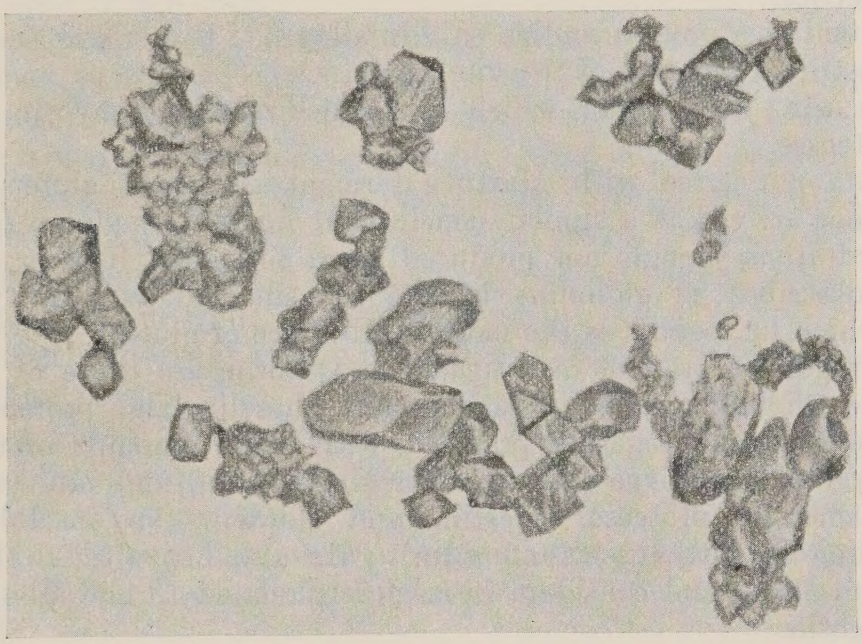


FIG. 11.—Crystallised vitellin of the oat kernel.—After Osborne.

insoluble proteose is, however, soluble in strong alcohol, and gives the sticky consistency to gluten; it corresponds to the two substances called gliadin and mucedin by Ritthausen.[3]

The existence of proteids soluble in strong, though probably not in absolute, alcohol, is a remarkable occurrence, and is not unique in vegetable physiology.

Martin considered that gluten does not pre-exist in wheat-flour, but is formed on the addition of water by a ferment action. This is supported by the fact that washing flour with water at a low temperature (2° C.) does not lead to the formation of gluten. The ferment, however, has not been separated, and Johannsen[4] has advanced certain facts that tell against the ferment theory and in favour of the pre-existence of gluten in the flour.

[1] *Am. Chem. Journ.*, Baltimore, vol. xiv. No. 8.

[2] *Brit. Med. Journ.*, London, 1886, vol. ii. p. 104.

[3] *Journ. f. prakt. Chem.*, Leipzig, Bd. lxxiv. S. 193, 384. For other observations on gluten, see Bouchardat, *Compt. rend. Acad. d. sc.*, Paris, tome xiv. p. 962; Taddei, *Gior. fisica di Brugnatelli*, vol. xii. p. 860; Gunsberg, *Journ. f. prakt. Chem.*, Leipzig, Bd. lxxxv. S. 213.

[4] *Ann. agronomiques*, Paris, tome xiv. p. 420.

Osborne[1] investigated the proteids of the oat and analysed three primary oat proteids, one soluble in alcohol, the second a globulin, and the third a proteid soluble in alkali only. From these, secondary proteids are obtained by mixing the ground oats with water; he regards the change as one produced by ferment activity.

In conjunction with Chittenden,[2] he worked out in a similar way the proteids of maize, and found there two globulins, one or more albumins, and a proteid soluble in alcohol. These differ in solubilities, coagulating points, and elementary composition; one of the globulins is a vitellin, the other a myosin. A small amount of proteose also present was regarded as artificially produced in the processes of analysis. The proteid soluble in alcohol is called zein; and it, like the globulins, is converted into an insoluble modification on admixture of the flour with water.

The proteids of flax seed[3] he found to be chiefly globulin, with smaller quantities of albumin, proteose, and peptone.

In wheat Osborne and Voorhees[4] describe five proteids:—

1. Gliadin; a proteid soluble in alcohol, and like gelatin in some of its other properties.
2. Glutenin; a proteid soluble only in alkalis.
3. Edestin; a globulin of the vitellin class.
4. Leucosin; an albumin, which Martin described as a myosin.
5. Proteoses.

They do not agree with Martin's ferment theory of gluten formation. O'Brien[5] has arrived at a similar conclusion; he regards gluten formation as due to hydration, though not produced by a ferment. The proteids in the flour he describes as globulins of the myosin and vitellin type, and a proteose which he regards as the mother substance of gluten.

Other vegetable proteids investigated by Osborne are those of the kidney bean[6] (two globulins called phaseolin and phaselin, and proteose); of the cotton seed (almost altogether proteose, with small amounts of edestin and insoluble proteid); of rye (gliadin, leucosin, edestin, and proteose); and of barley[7] (leucosin, proteose, edestin, and hordein, an insoluble proteid, corresponding to Ritthausen's mucedin). He also investigated the chemical nature of diastase, and considers it is closely related to the albumin he has named leucosin.

Researches on proteolytic ferments in plants.—Those in the papaw plant and in pine-apple juice are the best known, or most fully worked out.

Papain was the name given by Wurtz to the proteolytic ferment in the juice of the papaw plant.[8] The close similarity of its action to that of trypsin was shown by S. Martin.[9] The proteids in the juice are a globulin very like serum globulin, small quantities of an albumin, and proteoses of two kinds, with one of which the ferment appears to be closely associated (Martin).[10]

Bromelin.—This is the proteolytic ferment in pine-apple juice. Its existence was first noted by Marcano of Venezuela. It is made use of extensively in South America for the preparation of artificially digested

[1] *Am. Chem. Journ.*, Baltimore, vol. xiv. No. 3.
[2] *Ibid.*, vol. xiii. Nos. 7, 8, and 9; vol. xiv. No. 1. [3] *Ibid.*, vol. xiv. No. 8.
[4] *Ibid.*, vol. xv. No. 6; "Seventeenth Ann. Rep. Connecticut Agric. Expt. Station," Newhaven, 1893.
[5] *Ann. Botany*, Oxford, 1895, vol. ix. pp. 171, 503.
[6] *Journ. Am. Chem. Soc.*, N. Y., 1894, vol. xvi. p. 633.
[7] *Ibid.*, 1895, vol. xvii. p. 539. See also Osborne and Campbell on proteids of potato on conglutin and vitellin, on legumin and other proteids of the pea and vetch, *ibid.*, 1896 vol. xviii. No. 7.
[8] *Compt. rend. Acad. d. sc.*, Paris, 1879, p. 425; 1880, p. 1379.
[9] *Journ. Physiol.*, Cambridge and London, vol. v. p. 213.
[10] *Ibid.*, vol. vi. p. 336.

foods.[1] Its action has been studied by Chittenden[2] and his pupils. It is a ferment of intense activity, and acts well in neutral, acid, and alkaline solutions, especially at 60° C. The ferment itself is associated or identical with a proteose-like substance in the juice. The products of its action (proteoses and peptone) are like those of other proteolytic ferments.

I have alluded to these two ferments because they have formed the basis of very thorough investigations, not because they are in any way exceptional occurrences in the vegetable kingdom; as already stated, such ferments probably play an important part in all plants, by converting the insoluble proteid of the seed into the soluble nitrogenous substances of the sap.[3]

Proteids as Poisons.

The line between food and poison is easily crossed. When, a few years ago, the idea was first mooted that proteids may act as poisons, it was received with incredulity in many quarters; but there can now be no doubt that it is a fact.[4]

The best known of the vegetable proteid poisons are:—

1. Those contained in the seeds of jequirity (*Abrus precatorius*). Warden and Waddell[5] named the poisonous substance *abrin*. S. Martin[6] separated the two proteids—a globulin and a proteose—of which it is composed. The material is used as a drug to produce conjunctivitis.

2. The proteid associated with or identical with papain (S. Martin).

3. *Ricin*, the poisonous proteid in castor-oil beans.[7]

4. *Lupino-toxin* from *Lupinus luteus*.[8]

The most important of the animal proteid poisons are—

1. Snake poison.

2. Proteids in the serum of certain fishes (conger eel, muræna, etc.).[9]

3. Proteid poisons found in certain spiders,[10] and in the stinging apparatus of many insects.

4. Ordinary peptones and proteoses; 0·3 gr. of commercial peptone per kilog. of body weight is in dogs usually fatal, when injected into the blood.

5. Nucleo-proteids.—These were called tissue fibrinogens by Wooldridge, and cause intravascular clotting when injected into the blood (see "Coagulation of Blood").

6. Poisonous proteids produced by bacterial action. This subject has recently received much attention, and opens up the whole subject of toxins and antitoxins. To go into this matter thoroughly would

[1] *Bull. Pharm.*, Detroit, 1891, vol. v. p. 77.

[2] *Trans. Connect. Acad. Arts and Sc.*, New Haven, 1891, vol. viii.; *Journ. Physiol.*, Cambridge and London, vol. xv. p. 249.

[3] See further Green's papers already quoted; also J. R. Green, "On the presence of vegetable trypsin in the fruit of *Cucumis utilis* and other plants," *Ann. agronomiques*, Paris, tome xix. p. 508; Neumeister, *Ztschr. f. Biol.*, München, Bd. xxx. Another recent paper on the subject (J. Hjort, *Centralbl. f. Physiol.*, Leipzig, 1896, Bd. x. S. 192) shows that there are similar ferments in fungi.

[4] Nencki's opinion that poisonous proteids are more labile than other proteids can hardly be considered an explanation of this fact ("Ueber die labile Eiweissstoffe," *Wchnschr. f. Pharm.*, 1891, No. 29).

[5] "Non-Bacillar Nature of *Abrus* Poison," Calcutta, 1884.

[6] *Brit. Med. Journ.*, London, 1889, vol. ii. p. 184.

[7] Stillmark, *Pharm. Centr.-Bl.*, Leipzig, 1890, Bd. xxx. S. 650.

[8] *Schmidt's Jahrb.*, Leipzig, 1888, Bd. cciv. S. 10.

[9] Mosso, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xviii. S. 92.

[10] Kobert, *Sitzungsb. d. Dorpater naturforsch. Gesellsch.*, 1888; *Centralbl. f. d. med. Wissensch.*, Berlin, 1888, S. 544.

lead us too far into pathological regions. The exact nature of the toxalbumoses and their antitoxins is by no means settled, but has already been followed by important practical results in the way of treatment.

Snake poison.—The first group of proteid poisons in the foregoing list will furnish us with a typical example of the class, and it appears probable that, as the nature of the poison has been more thoroughly worked out in this than in most of the other cases, this will also form an important field of research in furnishing the key to the question of the nature of antitoxins; for protective inoculation has here been followed with considerable success (Calmette,[1] Fraser[2]).

The first investigation into the chemistry of snake poison of any importance was by Prince Lucien Buonaparte, on the poison of an adder, in 1843.[3] He found that the activity of the poison was associated with the portion precipitable by alcohol; and he gave the name *viperine* to this precipitate.

About 1860, Weir Mitchell[4] turned his attention to the subject, and he was the first to recognise that the toxic principle of the venom is albuminous in nature. He termed it *crotalin* in the case of the rattlesnake. From that time till 1886 (in conjunction with Reichert) he continued his work, and confirmed his general conclusion in the case of the North American snakes. About 1871 the Indian snakes received their share of attention, and the names of Sir Joseph Fayrer[5] and Lauder Brunton[6] are associated with valuable researches concerning the venom of the cobra, krait, and Indian viper. These observers dealt, however, with the object mainly from the point of view of the physiological action of the venom.

In 1883 Wall,[7] in 1886 Wolfenden,[8] and in 1892 Kanthack,[9] published important contributions to our knowledge of cobra poison, the improved methods of chemical physiology enabling them not only to identify the poison as a proteid, but to show that the variety of proteid present is principally proteose. Two observers have described poisons other than proteid in snake venom, viz. Gautier,[10] who regarded the venomous principle as alkaloidal; and Wynter Blyth,[11] who gave the name cobric acid to a crystalline substance which he separated from cobra venom, and which he asserted to be highly poisonous. Recent work has failed to substantiate these results, and such alkaloids as are present (and they are generally absent) are non-poisonous ones.

In their researches on the venom of the Australian black snake, C. J. Martin and M'G. Smith[12] determined positively the nature of the

[1] "Le Venin des Serpents," Paris, 1896.

[2] *Brit. Med. Journ.*, London, 1895, vol. i. p. 1309. The name given to the antitoxin contained in the serum of immunised animals is antivenine.

[3] See Sir J. Fayrer, *Proc. Med. Soc. London*, 1884.

[4] *N. Am. Med.-Chir. Rev.*, vol. v. p. 269; *Med. News*, Philadelphia, 1883; "Researches upon the Venoms of Poisonous Serpents," Smithsonian Contributions to Knowledge, 1886.

[5] *Rep. on san. improvements in India*, London, 1873, 1874.

[6] *Rep. on san. measures in India*, London, 1874; *Proc. Roy. Soc. London*, 1872–3, 1873–4, 1875 and 1878; Sir J. Fayrer, "Thanatophidia of India," London, 1872, and numerous papers by same author in *Edin. Med. Journ.*, and *Indian Med. Gaz.*, Calcutta, between 1868 and 1874.

[7] "Indian Snake Poisons, their Nature and Effects."

[8] *Journ. Physiol.*, Cambridge and London, vol. vii. pp. 327, 357, 365.

[9] *Ibid.*, vol. xiii. p. 372.

[10] *Bull. Acad. de méd.*, Paris, 1881.

[11] *Analyst*, London, 1876, vol. i.

[12] *Proc. Roy. Soc. New South Wales*, Sydney, July 3, Aug. 3, 1892; *Journ. Physiol.*, Cambridge and London, 1893, vol. xv. p. 380.

venom. By appropriate experiments they excluded micro-organisms, ferments, alkaloids, ptomaines, and crystalline acids.[1] They next showed that there are three proteids in the secretion; one, an albumin, is not irulent; but the other two, which are proteoses (proto- and hetero-proteose), are extremely poisonous. Their action is the same as that of the venom itself. They, like the venom, can be momentarily boiled without impairing their activity, but prolonged boiling for days destroys their virulence.

The action of the poison is local and general. The most marked local effect is œdema; the general symptoms in non-lethal doses consist of twitching and convulsions. A fatal dose kills within a few seconds or minutes. There is also a peculiar effect on the blood. More than a century ago, the Abbé Fontana[2] noticed that the blood of animals killed by viper bite remained fluid. Brainard,[3] writing more than forty years ago, states that when death occurs immediately, in animals bitten by rattlesnakes, the blood is found at the post-mortem examination to be clotted; but if some time elapses before the animal succumbs, the blood remains fluid in the vessels. The continued fluidity of the blood has since then been noted by numerous observers in the case of various snakes. These observations are explained by C. J. Martin's researches. He found that different doses produce different results. Immediately after the introduction of the venom, the coagulability of the blood increases, and this increase in the case of moderate or large doses (more than 0·0001 grm. per kilog. of body weight) culminates in intravascular clotting of greater or less extent. The injection of smaller doses produces a transient phase of increased coagulability, but after two minutes this is succeeded by a negative phase; the blood when drawn either fails to clot at all, or does so only after the lapse of several hours. The thrombosis occurs more readily in venous than arterial blood, and is frequently confined to the portal area. These results show a great resemblance between the action of the venom and that of nucleo-proteid. The effect of diminished coagulability is not unexpected, seeing that the principal substance in the venom is proteose, but the minuteness of the dose necessary is very striking and distinctive. The smallness of the dose suggests that the injected material does not itself contribute to fibrin-formation. It probably acts by producing disintegration of the cells of the endothelium of the blood vessels, or, according to Martin's later observations, of the red corpuscles; in either case the result would be liberation of nucleo-proteid material.

With regard to the question of how these poisonous proteoses are formed, Martin puts forward the following hypothesis: the cells of the venom gland exercise a hydrolysing agency on the albumins supplied them by the blood, the results of which influence are the poisonous proteoses found in the venom. A difference between the process and digestion by pepsin, or by anthrax bacilli, is that the hydration stops short at the proteose stage, and is not continued so as to form peptone, or simpler nitrogenous materials, like leucine, tyrosine, or alkaloids. Gland epithelium is certainly capable of exercising such a hydrolysing influence; the conversion of glycogen into sugar in the liver cells is one of the best known examples.

[1] A questionable trace of organic acid found did not possess toxic properties.
[2] Fontana, "Poisons," Trans. by J. Skinner, London, 1787.
[3] *Rep. Smithson. Inst.*, Washington, 1854.

The following table, somewhat altered from Sidney Martin,[1] illustrates the analogy between various hydrolysing processes, proteid being in all cases the material acted on.

Primary Agents.	Ferment.	Products.	
		Albuminous.	Nitrogenous but not Albuminous.
1. Epithelial cell of gastric gland	Pepsin.	Proteoses, peptone.	Brieger's pepto-toxin; a very doubtful basic substance.
2. Epithelial cell of pancreas	Trypsin.	Proteoses, peptone.	Leucine, tyrosine, lysine, arginine, aspartic acid, ammonia.
3. *Bacillus anthracis* . .	None yet found.	Proteoses, peptone.	Leucine, tyrosine, and an anthrax alkaloid.
4. *B. diphtheriæ* . . .	Ferment not named.	Proteoses.	Organic acid of doubtful nature.
5. Epithelial cell of snake's venom-gland	None yet found.	Proteoses.	Trace of organic acid.

Calmette[2] has worked out a table of the relative toxicity of venoms, as Roux and Vaillard have done for tetanus toxins, based on the ratio of lethal dose weight, subcutaneously injected, to body weight. He found the toxic value to be represented by the following numbers :—

Cobra	4,000,000
Hoplocephalus curtus	3,450,000
Pseudechis	800,000
Pelias berus	250,000

Martin places the toxic power of the two Australian venoms at—

Hoplocephalus	4,000,000
Pseudecis	2,000,000

This is a very high virulence; put in another way, it means that 0·00025 gr. of the one, and 0·0005 gr. of the other poison is sufficient to kill a rabbit weighing a kilogramme. The virulence of snake poison much exceeds that of most of the poisonous proteids of zymotic diseases, though it is about the same as the diphtheria toxin of Roux and Yersin.[3] The following table also gives the toxic value of anthrax toxin,[4] and toxopeptone[5] from cholera cultures calculated in the same way :—

Diphtheria toxin	4,000,000 (about)
Anthrax albumoses	80
Toxo-peptone	3,000

Animal Alkaloids.

Ptomaines and leucomaines.—The word *ptomaine* was originally employed to designate those putrefactive products of animal substances which give the reactions of vegetable alkaloids, and which are more or less poisonous. The similar substances formed by metabolic activity, either from lecithin or proteids,[6] are called *leucomaines.*

[1] Published in *Brit. Med. Journ.*, London, March 1892.
[2] *Ann. de l'Inst. Pasteur*, Paris, 1894, tome viii.
[3] Quoted by Sims Woodhead, "Bacteria and their Products," p. 307.
[4] Sidney Martin, *Rep. Med. Off. Local Gov. Bd.*, London, 1890–91.
[5] Petri, quoted by Vaughan and Novy, "Ptomaines and Leucomaines," p. 109.
[6] A discussion of the chemistry of the origin of alkaloids from proteids will be found in a paper by Latham, *Lancet*, London, 1888, vol. ii. p. 751.

The importance of the animal alkaloids was first brought into prominence in courts of law; the defence urged in certain notorious trials for murder, was that the alkaloid alleged to have been administered to the victim, or found in his stomach, really arose as the result of putrefactive changes occurring after death. It has, moreover, been demonstrated that alkaloids existing in different forms of putrefying food, produce poisonous symptoms. Sausages made with bad meat, certain forms of stale milk and cheese,[1] mussels and other shell fish,[2] at certain seasons of the year, produce serious symptoms in those who partake of them.

It has further been supposed that, in many cases of disease, the poison formed by bacteria in the body, and which produces the symptoms of the disease, is of an alkaloidal nature. The probability that cholera is caused by an alkaloid was first pointed out by Lauder Brunton,[3] from the similarity of the symptoms to those produced by muscarine poisoning. Two alkaloids at least have, in fact, been discovered in cholera, and in cultures of Koch's comma bacillus, and have been named cadaverine and putrescine, but they cannot be the actual poisons in cholera, because they are not markedly toxic. The same two alkaloids are found in the urine and fæces in totally different pathological conditions, namely, cystinuria,[4] and pernicious anæmia.[5]

Alkaloids in animal tissues were first described by Dupré and Bence Jones;[6] the substance they separated they called "animal quinoidine"; about the same time, Marquardt[7] obtained an alkaloid from a corpse, and named it "septicine." Schmidt[8] and Panum[9] obtained a substance they named sepsine from septic fluids, and they considered that it was the cause of septicæmia. Later, prominent workers at the subject have been, Selmi,[10] Gautier,[11] and Brieger;[12] to Brieger we owe the best methods of obtaining these substances in a state of purity. Brieger separated some alkaloids with such powerfully toxic properties, that he named them toxins; these include typhotoxine (from cases of typhoid fever), and tetanine[13] (from cases of tetanus).

All poisons produced by bacteria are, however, not necessarily ptomaines. In fact, many of the toxins and antitoxins have been shown to owe their power, at one time ascribed to ptomaines, to the tox-albumoses or poisonous proteids (see "Proteids as Poisons," p. 55).

A few details concerning the principal animal alkaloids may be added.

Parvoline ($C_9H_{13}N$).—This was first separated from the putrid flesh of the mackerel and horse. It is an oily base, but its chloroaurate and chloroplatinate are crystalline (Gautier).[14]

Hydrocollidine ($C_8H_{13}N$, boiling point 210° C.), and

Collidine ($C_8H_{11}N$) have been obtained from flesh, from putrid ox pancreas, and from gelatin. Nencki considers collidine to be isophenylethylamine, $C_6H_5—CH\begin{matrix}CH_3\\NH_2\end{matrix}$. These three bases are all highly toxic.

[1] Vaughan separated an alkaloid, which he named tyrotoxicon, from certain forms of bad cheese, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. x. S. 146.

[2] Mytilotoxin is the alkaloid separated from mussels by Brieger.

[3] *Rep. Brit. Ass. Adv. Sc.*, London, 1873.

[4] Baumann and Udranszky, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 562.

[5] Hunter, *Lancet*, London, 1888, vol. ii. p. 654.

[6] *Proc. Roy. Soc. London*, vol. xv. p. 73; *Ztschr. f. Chem.* 1866, S. 348.

[7] Schuchardt in Maschka's "Handb. f. ger. Med.," Bd. ii. S. 60.

[8] Inaug. Diss., Dorpat, 1869.

[9] *Virchow's Archiv*, Bde. xxvii., xxviii., and xxix.

[10] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xi. S. 808.

[11] Numerous papers; see especially *Bull. Soc. chim.*, Paris, tome xi. p. 6.

[12] Brieger, "Die Ptomaine," 1885, part i.; 1885, part ii.; 1886, part iii.

[13] Brieger, *Berl. klin. Wchnschr.*, 1888, No. 17.

[14] *Loc. cit.*

Neuridine ($C_5H_{14}N_2$) is a constant product of putrefaction of proteids. It is broken up by sodium hydrate into dimethylamine and trimethylamine (Brieger). Isomeric with this, though differing from it in the solubility of its salts, is *saprine.*

Cadaverine, a third isomeride, belongs to the diamine group, and in constitution is pentamethylenediamine (Ladenberg).[1]

Putrescine ($C_4H_{12}N_2$) is also a diamine, being tetramethylenediamine. It usually accompanies cadaverine, but as a rule makes its appearance later.[2]

All the above are free from oxygen; the remainder are oxygenated.

Neurine ($C_5H_{13}NO$) and *choline* ($C_5H_{15}NO_2$) are constant products of cadaveric putrefaction, and their constitution has been described on p. 21. They are toxic, and derive additional interest from their close relationship to *muscarine* ($C_5H_{13}NO_2$), the alkaloid of the poisonous mushroom, *Agaricus muscarius.*[3] Muscarine was discovered by Schmiedeberg and Koppe.[4] Schmiedeberg and Harnack[5] obtained it also by oxidising choline with nitric acid. Brieger found it in putrid fish, and it occurs in several vegetables.[6]

The natural alkaloid is probably not identical, but isomeric with that prepared by the oxidation of choline;[7] more recently an alkaloid, with all the properties of the muscarine of plants, has been prepared artificially from monochloracetal and trimethylamine.[8] The constitutional formula of muscarine is—

$$N\begin{cases}(CH_3)_3\\ CH_2—C\overset{H}{\underset{O}{}}\\ OH\end{cases}$$

and it is the aldehyde of the non-toxic betaine (trimethylglycocine).[9]

Choline, neurine, and muscarine are all toxic; and are antagonistic to atropine, so far as relates to their action on the heart and glandular system.[10]

Gadinine ($C_7H_{16}NO_2$) is a less toxic alkaloid, which is mixed with the muscarine obtained by Brieger from putrefying cod-fish.

Mytilotoxine ($C_6H_{15}NO_2$) is the active agent in mussel poisoning.

Typhotoxine ($C_7H_{17}NO_2$) is obtained from cultures of the typhoid bacillus, and was regarded by Brieger as the chemical poison in typhoid fever.

Tetanine ($C_{13}H_{22}N_2O_4$) is, or was supposed to be, the toxin in cases of tetanus (Brieger).

Gautier completes his list of animal alkaloids by including a number of substances of the uric acid group (adenine, guanine, xanthine, hypoxanthine, etc.), and of the creatinine group (creatinine itself, and certain substances separated from muscle, which are termed xanthocreatinine, $C_5H_{10}N_4O$,

[1] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xix. S. 2585.

[2] Brieger, *Berl. klin. Wchnschr.*, 1887, No. 44; Boeklisch, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xx. S. 1441; Baumann and Udranszky, *ibid.*, Bd. xxi. S. 2938; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 562; Brieger and Stadthagen, *Virchow's Archiv*, Bd. cxv. Heft 3.

[3] The *Agaricus muscarius* also contains a considerable amount of a non-toxic alkaloid, *amanitine*, Neumeister, "Physiol. Chem.," Bd. i. S. 71. [4] "Das Muscarin," Leipzig, 1869.

[5] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1876, Bd. vi. S. 101.

[6] Such as *Beta vulgaris*, and the seeds of vetches and cotton. E. Schulze, *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 140; and 1892, Bd. xvi. S. 205.

[7] Boehm, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1885, Bd. xix. S. 87.

[8] Berlinerblau, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1884, Bd. xvii. S. 1139.

[9] Found in *Beta vulgaris*; betaine has been also synthetically prepared from monochloracetic acid and trimethylamine:—

$$CH_2Cl.COOH + N(CH_3)_3 + H_2O = N\begin{cases}(CH_3)_3\\ CH_2—COOH\\ OH\end{cases} + HCl.$$

[10] The fall of blood pressure produced by choline and neurine is of cardiac origin (Mott and Halliburton, "Proc. Physiol. Soc.," Feb. 1897, p. xviii., in *Journ. Physiol.*, Cambridge and London, vol. xxi.).

crusocreatinine, $C_5H_8N_4O$, and amphicreatinine, $C_9H_{19}N_7O_4$). These leucomaines are regarded by Gautier, Bouchard, Pouchet, and others, as feebly toxic products of metabolism, from which the organism is normally freed by excretion, or by destructive oxidation; it has been suggested that their retention in the body may be the cause of certain obscure pathological conditions. The poisonous properties of normal urine are regarded by some as due to alkaloids of this nature, while others (Stadthagen) look upon the inorganic, especially the potassium, salts of urine, as the toxic agents.[1]

Compound Proteids.

The compound proteids are compounds of albuminous substances with other materials, which are as a rule also of a complex nature.

They may be divided into the following groups:—

1. *Respiratory pigments.* — The most important of these are *hæmoglobin* and its compounds, *chlorocruorin*[2] (found in the blood of certain worms), and *hæmocyanin*[3] (found in the blood of many molluscs and crustacea). Hæmoglobin and chlorocruorin are compounds of proteids, with an iron-containing pigment. Hæmocyanin contains copper in its molecule. *Turacin*, the red pigment in the feathers of certain birds (plantain-eaters), also contains copper, and though not respiratory in function, should probably be included in the same group of substances.[4] Hæmoglobin with its derivatives and allies will be considered in a separate article.

2. *Gluco-proteids.*—Compounds of proteids with members of the carbohydrate group. This class includes mucins, mucoids, hyalogens and phospho-gluco-proteids.

3. *Nuclein.*—Compounds of proteid with phosphoric acid, or with nucleic acid.

4. *Nucleo-proteids.*—Compounds of proteid with nuclein.

5. *Lecith-albumins.*—Compounds of proteid with lecithin.

We may consider the last four groups in detail.

The gluco-proteids.—The gluco-proteids are mostly free from phosphorus (mucins, mucoids, and hyalogens), but some contain phosphorus (phospho-gluco-proteids).

Mucins.—The mucins are colloid, viscous substances of acid nature, soluble in alkalis, but precipitable from such solution by acetic acid. On boiling with dilute mineral acid they yield a substance which reduces Fehling's solution. They are found in the secretion of mucous glands, including the mucous salivary glands, and of slimy animals like

[1] For the principal papers on alkaloidal substances in urine, see Baumann and Udranszky, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 562; Stadthagen and Brieger, *Virchow's Archiv*, Bd. cxv.; Stadthagen, *Ztschr. f. klin. Med.*, Berlin, 1889, Bd. xv. Hefte 5 and 6; Pouchet, *Compt. rend. Acad. d. sc.*, Paris, tome xcviii. p. 1360; Bouchard, *ibid.*, tome cii. pp. 669, 727, 1127; Griffiths, *ibid.*, tomes cxiii., cxiv., and cxv.; Gautier, *Bull. Acad. de méd.*, Paris, 1886, tome xix. A very complete bibliography will be found in Huppert-Neubauer's "Analyse des Harns," 9th edition, p. 241.

[2] Quatrefages, see Gamgee, "Physiological Chemistry," vol. i. p. 131; Krukenberg, "Vergl. physiol. Studien," 2te Reihe. Abth. 1, S. 87; Lankester, *Journ. Anat. and Physiol.*, London, vol. ii. p. 114; vol. iii. p. 119.; MacMunn, *Quart. Journ. Micr. Sc.*, London, Oct. 1885.

[3] Fredericq, *Bull. Acad. roy. de méd. de Belg.*, Bruxelles, 1878, Sér. 2, tome xlvi. No. 11; Halliburton, *Journ. Physiol.*, Cambridge and London, vol. vi. p. 300. In the latter paper numerous references to other writers will be found.

[4] A. H. Church, *Proc. Roy. Soc. London*, 1869, vol. xvii. p. 436; *Phil. Trans.*, London, 1869, vol. clix. p. 627; 1892, vol. clxxxiii. p. 511; A. Gamgee, *Proc. Roy. Soc. London*, 1896, vol. lix. p. 339.

snails.[1] A mucinogen is found in the investment around frogs' eggs;[2] it is also the most important constituent of the intercellular or ground substance of connective tissues, and has been especially investigated in the jelly-like connective tissues (vitreous humour,[3] Whartonian jelly[4]), and in tendon.[5]

Elementary analysis of different mucins has given different results, as will be seen from the following table:—

	Snail Mucin.	Tendon Mucin.		Submaxillary Mucin.	
	Hammarsten.[6]	Loebisch.[7]	Chittenden.[8]	Hammarsten.[9]	Obolensky.[10]
C . .	50·32	48·3	48·26	48·84	52·31
H . .	6·84	6·44	6·49	6·80	7·22
N . .	13·65	11·75	11·51	12·32	11·84
S . .	1·75	0·81	2·31	0·84	...
O . .	27·44	32·70	31·43	31·20	28·63

The mucins thus contain less carbon, and considerably less nitrogen, than proteids.

Decomposition products of mucin.—By the action of superheated steam, a carbohydrate is split off from mucin, which was called animal gum by Landwehr.[11] He assigns to it the formula $(C_6H_{10}O_5)$. By the action of dilute mineral acids this is converted into a reducing but non-fermentable sugar or gummose $(C_6H_{12}O_6)$. The gum-like substance obtained from submaxillary mucin contains nitrogen.[12] The sugar obtained from tendon mucin by Chittenden yielded an osazone melting at 160°, and resembled that obtained from pentoses. F. Müller[13] has investigated the mucin of sputum. He found it yielded as much as 25 to 32 per cent. of a reducing substance; this is not a pentose, but is probably glucosamine.

[1] Eichwald, *Ann. d. Chem.*, Leipzig, Bd. cxxxiv.

[2] Giacosa, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vii. S. 40; Hammarsten, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxxvi.; Wolfenden, *Journ. Physiol.*, Cambridge and London, vol. v. p. 91.

[3] R. A. Young, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 325; C. Th. Mörner, *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xviii. S. 245. References to previous literature will be found in these papers. Young arrived at the conclusion that the principal substance in vitreous humour is mucinogen, not mucin.

[4] Jernström, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1889, Bd. x. S. 34. Young, *loc. cit.*, separated two mucins from the Whartonian jelly, one soluble, the other insoluble in excess of acetic acid.

[5] Rollett, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. xxxix. S. 308, Stricker's "Handbuch," Bd. i. S. 72. Loebisch, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. x. S. 40; Chittenden and Gies, *Journ. Exper. Med.*, Baltimore, 1896, vol. i. p. 188.

[6] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxxvi. [7] *Loc. cit.*

[8] *Loc. cit.* The high percentage of sulphur found is attributed by Chittenden to proteid impurities.

[9] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii.

[10] *Arch. f. d. ges. Physiol.*, Bonn, Bd. iv. S. 336. Probably Obolensky's preparation was not so pure as Hammarsten's.

[11] *Ztschr. f. physiol. Chem.*, Strassburg, 1881, Bd. viii. S. 124, 199; *Arch. f. d. ges. Physiol.*, Bonn, Bde. xxxix. and xl.

[12] Hammarsten, "Physiol. Chem.," 3rd German edition, p. 39.

[13] *Centralbl. f. Physiol.*, Leipzig, 1896, Bd. x. S. 480; *Sitzungsb. d. Gesellsch. z. Beförd. d. ges. Naturw. zu Marburg*, 1896, No. 6.

By the action of dilute mineral acids on mucin, this reducing substance, whatever its exact nature is, is also obtained, together with syntonin and proteose-like materials, from the proteid part of the mucin molecule. Strong acids lead to the formation of leucine, tyrosine, and levulinic acid (Landwehr). Strong alkalis lead to the formation of similar products; but weak alkalis, like lime water, have no effect on tendon mucin, though they readily break up submaxillary mucin (Loebisch). There is a good deal of difference among the mucins in their solubilities in acid and alkaline solutions. Obolensky obtained pyrocatechin by boiling submaxillary mucin with caustic soda; but I have not succeeded in getting it from connective tissue mucin.[1]

The putrefactive products of mucin are similar to those obtained from proteids.

Mucoids or mucinoids.—These are mucin-like substances, which differ from the true mucins either in being non-precipitable from alkaline solutions by acetic acid, or in being readily soluble in excess of acetic acid. The designation was originally given to this class by Hammarsten, and includes the following substances:—

1. The mucin from vitreous humour.
2. The mucin from cartilage—*chondro-mucoid* (see "Cartilage").
3. The mucin from cornea—*cornea-mucoid*.[2]
4. *Pseudo-mucin*; the colloid-like substance often found in ovarian fluids, and previously known as paralbumin and metalbumin.[3]
5. A similar mucoid, sometimes found in ascitic fluid.[4]
6. *Ovomucoid*, a mucoid found in white of egg. This was first studied by Neumeister,[5] who called it pseudo-peptone, then by Salkowski,[6] and finally by C. T. Mörner,[7] who identified it as a mucoid.
7. *Paramucin*, a substance found sometimes in ovarian cysts, differing from pseudomucin in reducing Fehling's solution without previous treatment with acids (K. Mitjukoff).[8] Leathes,[9] who has worked at this substance under Drechsel's supervision, finds that the reducing substance yields no osazone; that on decomposition it yields sulphuric acid, and thus resembles chondromucoid; and on treatment with hydrochloric acid it gives off carbonic anhydride. Its nature is still uncertain.

Hyalogens.—The term hyalin was originally applied to the principal constituent of the wall of hydatid cysts.[10] Krukenberg[11] extended the name to allied substances obtainable from other animal structures. In the natural state these substances are insoluble, and are termed hyalogens; by the action of alkalis or superheated water they are converted into the soluble hyalins. Neossidin is the hyalin obtained from neossin,[12] the chief constituent of the edible bird's nest. Chondrosidin and chondrosin are the hyalin and hyalogen respectively obtained from the sponge, *Chondrosia reniformis*,

[1] See also Young, *loc. cit.*

[2] C. T. Mörner, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 213.

[3] Hammarsten, "Lehrbuch d. physiol. Chem.," 3rd German edition, S. 366. See also Hammarsten, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xi. S. 11; Landwehr, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vii. S. 118.

[4] Hammarsten, *ibid.*, 1891, Bd. xv. S. 202.

[5] *Ztschr. f. Biol.*, München, Bd. xxvii. S. 309.

[6] *Centralbl. f. d. med. Wissensch.*, Berlin, 1893, Nos. 31 and 43.

[7] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 525.

[8] Inaug. Diss., Berlin, 1895.

[9] Communication to Physiological Society, London, Oct. 17, 1896 (not published).

[10] Lücke, *Virchow's Archiv*, Bd. xix. S. 189.

[11] *Ztschr. f. Biol.*, München, Bd. xxii. S. 261.

[12] The word "neossin" is Mulder's, *Bull. des sc. phys. in Nederlande*, 1838, S. 172; Green, *Journ. Physiol.*, Cambridge and London, vol. vi. p. 40, pointed out the resemblance of the nest substance to mucin.

spirographidin and spirographin from the skeletal tissues of the worm *Spirographis*. Krukenberg obtained a hyalogen also from the tubes of *Onuphis tubicola*, another from the membrane of Descemet and lens capsule (membranin, C. T. Mörner),[1] and another from hyaline cartilage (now called chondroitin-sulphuric acid, see "Cartilage"). These substances are all, like the mucins and mucoids, decomposed by acids with the formation of a reducing substance. They differ from the mucins in some of their solubilities, but it is doubtful whether they should be classed apart from the mucoids.

Phospho-gluco-proteids.—These substances not only yield a reducing carbohydrate or carbohydrate-like body, like the mucins and mucoids, but on gastric digestion they leave a residue of pseudo-nuclein, a substance which, like nuclein, contains phosphorus. Pseudo-nuclein does not, however, yield bodies of the xanthine group, on further decomposition, as do true nucleins. Among these substances are the following:—

(*a*) *Ichthulin*, a substance separated from the eggs of the carp by Walter,[2] and at first supposed to be identical with vitellin.

(*b*) *Helico-proteid*, secreted by the glands of the snail (*Helix pomatia*), and separated by Hammarsten.[3] By the action of alkalis a levorotatory carbohydrate (animal sinistrin) is split off; a dextrorotatory reducing sugar is obtained by the use of dilute mineral acids.

(*c*) The principal constituent of the cells of the pancreas is a complex nucleo-proteid which Hammarsten[4] considers to be identical with trypsin; by boiling this, it is split into coagulated proteid and a phospho-gluco-proteid. The sugar which this substance gives, on treatment with dilute acids, is probably a pentose (see p. 3).

Kossel and his pupils have also obtained reducing sugar-like substances from yeast nuclein.

In concluding the subject of the gluco-proteids, it may again be mentioned that Pavy regards all the common proteids (casein excepted) as having a glucoside constitution (see p. 30). Whether this be so or not, the fact insisted upon by Pavy that a carbohydrate may be obtained by hydrolytic decomposition of proteids has been confirmed by other observers. Thus K. Mörner obtained from serum globulin a reducing substance on treatment with hydrochloric acid, which, like Pavy's, is optically inactive; but failed to get such a substance from purified myosin, vitellin, crystallin, serum albumin, and egg albumin. He got it from fibrin, but considered that it was due to carbohydrate in entangled blood corpuscles.[5] I myself was at one time of opinion that Pavy's results, which were principally obtained with egg-white, were due to the admixture of the pure albumin with a mucoid (ovomucoid, which exists to the extent of 10 per cent. in egg-white); but I learn from Dr. Pavy that his method of preparing coagulated egg-white would exclude any large admixture of this kind. Pavy's work, moreover, has been recently confirmed by N. Krawkow.[6] He found egg-white difficult to obtain free from ovomucoid, but the purest products he obtained always yielded a reducing substance, which gave a crystalline osazone (melting at 183° to 185° C.; Pavy gives 189° C.). This reducing substance he regards as a carbohydrate, though he does not commit himself as to its identity. He, however, never found pentoses, nor did he find that the gastric digestion of egg albumin yielded any carbohydrate. The same carbohydrate was obtained from acid albumin, alkali albumin, albumose, peptone, fibrin, serum albumin, serum globulin, and lact-albumin. Casein, vitellin, gelatin, and nucleo-proteid from peas gave a negative result. Albumin from peas yielded an osazone rather

[1] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 213.
[2] *Ibid.* Bd. xv.
[3] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxxvi.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xix. S. 19.
[5] *Centralbl. f. Physiol.*, Leipzig, Bd. vii. S. 581.
[6] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxv. S. 281.

different in its characters from the one just described. H. Weydemann[1] has also confirmed Pavy's work; he considers that the material in the proteid that yields the reducing substance is identical with Landwehr's animal gum.

The nucleins.—Lauder Brunton[2] described the nuclei of the red corpuscles of birds as consisting of a mucin-like substance. Plósz,[3] however, found that, though the material in question resembled mucin in its solubility in alkalis, and precipitability by acids, it was not mucin, as it contains a high percentage of phosphorus. About the same time Miescher[4] separated a similar phosphorus-rich substance from the nuclei of pus corpuscles; the pus was subjected to gastric digestion, and the nuclein alone remained undissolved. Later, Miescher[5] prepared a similar substance from the spermatozoa of different animals, and from egg-yolk; Hoppe-Seyler,[6] Kossel,[7] and Loew[8] from yeast, Plósz[9] from the liver, Jaksch[10] and Geoghegan[11] from brain, Lubavin[12] from cows' milk, and Worm-Müller[13] from egg-yolk.

It was soon surmised that nuclein is not a single substance, because the different nucleins vary in their solubilities, and even in their composition. Miescher's nuclein from spermatozoa, for instance, contained no sulphur. Of recent years our knowledge of the nucleins has been considerably advanced by Kossel,[14] Liebermann, and others.

It has long been known that metaphosphoric acid is a precipitant of albumin. Liebermann[15] examined this precipitate and found that it gave many of the reactions of nuclein. He therefore came to the conclusion that nuclein is simply a compound of albumin with phosphoric acid. Malfatti[16] carried this idea still further, for he found that, by fractional precipitation with different amounts of phosphoric acid, he was able to obtain a chain of nucleins with different amounts of phosphorus in each, and with varying solubilities, corresponding closely with those obtainable from nuclei.

Pohl,[17] however, very soon showed that Liebermann's precipitate differs from true nuclein (*i.e.* the nuclein from nuclei) in the fact that substances of the xanthine group are not obtainable from it on decomposition, and Kossel[18] has contested Liebermann's and Malfatti's views chiefly on the same grounds.

Kossel divides the nucleins into two groups. The first is that of the true nucleins. These are obtainable from nuclei; they yield on decomposition the xanthine bases—hypoxanthine, adenine, and other substances of the same group. The second class of nucleins may be called pseudo-nucleins, and include those obtainable from milk, egg-yolk,

[1] Inaug. Diss., Marburg, 1896; *Centralbl. f. Physiol.*, Leipzig, 1897, Bd. x. S. 749.
[2] *Journ. Anat. and Physiol.*, London, 2nd series, vol. iii. p. 91.
[3] Hoppe-Seyler, "Med. Chem. Untersuch.," 1871, Heft 4, S. 460. [4] *Ibid.*, S. 441.
[5] *Verhandl. d. naturf. Gesellsch. in Basel*, 1874, Heft i.
[6] "Med. Chem. Untersuch.," Bd. iv. S. 500.
[7] *Ztschr. f. physiol. Chem.*, Strassburg, Bde. iii. and iv.
[8] *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxii. [9] *Ibid.*, Bd. vii.
[10] *Ibid.*, Bd. xiii. [11] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. i.
[12] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. x. S. 2237.
[13] *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. viii. S. 190.
[14] *Ztschr. f. physiol. Chem.*, Strassburg. Numerous papers from Bd. viii. to present time.
[15] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxi. S. 598.
[16] *Ber. d. naturw.-med. Ver. in Innsbruck*, 1891–92, Bd. xx.; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvi. S. 69; xvii. S. 8.
[17] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 292.
[18] *Verhandl. d. physiol. Gesellsch.*, Berlin, Oct. 21, 1892 (in *Arch. f. Physiol.*, Leipzig, 1892).

and Liebermann's artificial nuclein. Altmann[1] showed that the nitrogenous bases just alluded to originate from a complex organic acid, which he termed *nucleic acid*, and that the true nucleins differ from one another in the relative quantities of proteid and nucleic acid which they contain. Nucleic acid is free from sulphur, and is in fact identical with Miescher's nuclein from spermatozoa. Miescher's formula for this sulphur-free material was $C_{29}H_{49}N_9P_3O_{22}$. Kossel's is $C_{30}H_{32}N_9P_3O_{17}$. More recent investigations by Miescher,[2] which were not published until quite recently (after his death), by Schmiedeberg, led him to adopt the formula $C_{40}H_{54}N_{14}O_{17}(P_2O_5)_2$ for nucleic acid. He further considered that in the spermatozoa, this acid is united to protamine. An examination of a preparation of nucleic acid, made from yeast by Altmann, showed that here the formula was $C_{40}H_{59}N_{16}O_{22}(P_2O_5)_2$ (see further under "Spermatozoa"). Nucleic acid does not give the proteid reactions. The relative amount of nucleic acid in different nucleins can be roughly determined by micro-chemical reactions with aniline dyes, nucleic acid having a great affinity for basic dyes like methyl-green.[3]

Hoppe-Seyler's classification of nucleins is the following:—

1. Nucleins like those found in spermatozoa, which contain no proteid, but consist only of nucleic acid.

2. The true nucleins, those found in cell nuclei. They yield proteid, xanthine or alloxuric bases (hypoxanthine, xanthine, guanine, adenine), and phosphoric acid. Those richest in nucleic acid occur in the chromatic fibres of the nucleus; poorer in nucleic acid are the nucleins which occur in the nucleoli (*e.g.* pyrenin), and which constitute the chief bulk of the substance called plastin by histologists; these are comparatively insoluble in alkalis. They form numerous links in a chain which passes insensibly into the group of the nucleo-proteids.

3. The para-nucleins (or pseudo-nucleins); these are the nucleins obtainable from nucleo-proteids (caseinogen, vitellin, cell nucleo-proteids). They yield (like Liebermann's artificial nuclein) no nitrogenous bases, but only proteid and phosphoric acid on boiling with water or dilute acid. The nucleo-proteids of cell protoplasm can only be provisionally included in this group; they contain so little nuclein, that even if xanthine bases were obtained from these (and the point does not seem to have been thoroughly investigated yet) the small yield might escape detection. The nucleo-proteid from muscle yields some of these bases (see "Chemistry of Muscle").

There are at least four nucleic acids. They are compounds of an acid with various bases, such as adenine, hypoxanthine, guanine, and xanthine. They differ in the amount and character of the bases, and in the acid with which these bases are combined. That from the thymus is called adenylic acid (from the fact that its chief base is adenine). This, when heated with sulphuric acid, yields a crystalline substance called thymin[4] ($C_5H_6N_2O_2$), cytosine, ammonia, levulinic acid, formic acid, and phosphoric acid. The yield of cytosine, a new crystalline base ($C_{21}H_{30}N_{16}O_4 + 5H_2O$) amounts to about 2 per cent. of the nucleic acid employed. The presence of levulinic acid among the products of decomposition is significant, and shows that adenylic acid contains a carbohydrate group. This agrees with previous

[1] *Arch. f. Physiol.*, Leipzig, 1889, S. 524. See also Kossel, *ibid.*, 1891.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, Bd. xxxvii. S. 100.

[3] For a criticism of these microchemical methods, see Heine, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxi. S. 494.

[4] Kossel and Neumann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxvi. S. 2753.

researches of Kossel, who obtained a carbohydrate from the nucleic acid of yeast.[1]

Kossel and Neumann[2] have further shown that adenylic acid yields also a new acid called thymic acid, precipitable as a barium salt ($C_{16}H_{23}N_3P_2O_{12}Ba$). The acid is readily soluble in cold water, and differs from nucleic acid in not being precipitated by mineral acids.[3]

Researches such as these show how complicated the subject is, and how much yet remains to be discovered, especially regarding the nuclein acids. The nuclein bases are comparatively simple, and the principal ones may be arranged in two groups:—

Adenine has the formula $C_5H_5N_5$; on heating it with sulphuric acid, NH is replaced by O, and *hypoxanthine* is formed:—

$$\underset{\text{(adenine)}}{C_5H_4N_4.NH} + \underset{\text{(water)}}{H_2O} = \underset{\text{(hypoxanthine)}}{C_5H_4N_4O} + \underset{\text{(ammonia)}}{NH_3}$$

Both substances contain a radicle, $C_5H_4N_4$, which Kossel terms *adenyl*; adenine is its imide, hypoxanthine its oxide. The following equation shows a similar relationship between *guanine* and *xanthine*:—

$$\underset{\text{(guanine)}}{C_5H_4N_4O.NH} + \underset{\text{(water)}}{H_2O} = \underset{\text{(xanthine)}}{C_5H_4N_4O_2} + \underset{\text{(ammonia)}}{NH_3}$$

On comparing the formulæ of hypoxanthine and xanthine with uric acid ($C_5H_4N_4O_3$), we see their close relationship. Leaving aside other possible ways in which uric acid is undoubtedly formed in the organism, we have here a way in which uric acid may arise by oxidation from the nuclein bases, and thus ultimately from the nuclei of cells.[4] The name "alloxuric bases" for these substances was suggested by Krüger and Wulff.[5] They are often spoken of as the "xanthine bases."

The nucleo-proteids.—These are compounds of nuclein with proteids. The amount of proteid matter is large, and the substances in question give the reactions of proteids, and in their solubilities approach very nearly to the globulins. On gastric digestion the nuclein they contain is left as an insoluble residue, but on pancreatic digestion a good deal of the nuclein is dissolved, and presumably, when this occurs in the body, is absorbed.[6]

Hammarsten divides the nucleo-proteids into two classes; the first, to which he restricts that name, yields true nuclein on gastric digestion; the other class he calls nucleo-albumins; these yield pseudo-nuclein on gastric digestion, and include caseinogen and vitellin. In addition to these, there are the phospho-gluco-proteids, which have already been described (p. 64).

Nucleo-proteids, using the term in the widest sense, are obtain-

[1] Kossel and Neumann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxvii. S. 2215.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxii. S. 74.

[3] It was later obtained from spermatozoa nuclein (Kossel, *ibid.*, p. 188). Milroy (*ibid.*, 1896, Bd. xxii. S. 307) states that the precipitate formed on adding nucleic acid to a solution of albumin resembles true nuclein in its characters; whereas the precipitate produced by thymic acid is somewhat similar to para-nuclein or pseudo-nuclein.

[4] This subject has been specially taken up by Horbaczewski (*Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. c.), who has pointed out the close relationship between uric acid formation and leucocytosis. Diet increases uric acid formation by leading to an increase of leucocytes, or possibly, as some recent investigators think, the increase is chiefly due to the nuclein in the food (Weintrand, *Chem. Centr.-Bl.*, Leipzig, 1895, Bd. ii. S. 54, 234, 310). See also Umber, *Ztschr. f. klin. Med.*, Berlin, 1896, Bd. xxix. S. 174; Camerer, *Ztschr. f. Biol.*, München, 1896, Bd. xxxiii. S. 139.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xx. S. 176.

[6] Popoff, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 533; Gumlich, *ibid.*, S. 508.

able from the nuclei and protoplasm of cells. They appear to be the most abundant of the proteid materials obtainable from cells. Nucleo-histon is the name of one of these separated from the thymus by Kossel and Lilienfeld.[1] The latter gives its percentage composition as C, 48·46; H, 7; N, 16·86; P, 3·025; S, 0·7; O, 23·95. The high percentage of phosphorus given here has never been obtained by me, from the numerous nucleo-proteids I have prepared and examined from the thymus and other organs. Details of these will be given under the heads of the various organs in question. In my own analyses, the amount of phosphorus rarely has exceeded 1 per cent.

Nucleo-histon appears to be identical with the tissue fibrinogen of Wooldridge, but this included a variable amount of lecithin. Other forms of the same substance have been called cytoglobin and preglobin by A. Schmidt.[2] Wooldridge prepared his tissue fibrinogens from cellular structures, such as thymus and testis. The gland is finely minced and extracted with water for twenty-four hours. Weak acetic acid is then added to the decanted extract, and after some hours the precipitated nucleo-proteid falls to the bottom of the vessel. This is the method used by Lilienfeld in the manufacture of nucleo-histon. Another method, which I have largely used, is to grind up the finely minced organ with about an equal volume of sodium chloride in a mortar. The resulting viscous mass (originally called hyaline substance by Rovida) is poured into excess of distilled water. The nucleo-proteid rises in strings to the surface of the water, where it may be skimmed off.

Prepared by either method, the nucleo-proteid may be dissolved in 1 per cent. sodium carbonate solution. This solution injected intravascularly in small doses in dogs produces a hindering of the coagulation of the blood (Wooldridge's negative phase). In larger doses it produces intravascular coagulation.[3]

The lecithin found associated with Wooldridge's tissue fibrinogens is variable in quantity, and does not appear to be organically united to them. After its removal the nucleo-proteids continue to exercise their most distinctive physiological characteristic, in producing intravascular clotting.[4]

In connection with nucleins and nucleo-proteids, it should be mentioned that many of them contain iron, and, according to Bunge,[5] constitute in foods the normal supply of iron to the body; in this sense he has called them hæmatogens. The composition of hæmatogen from egg-yolk he gives in percentages, which may be compared with the composition of nuclein from yeast, as follows:—

	Hæmatogen.	Nuclein from Yeast.
C . . .	42·11	40·81
H . . .	6·08	5·38
N . . .	14·73	15·98
O . . .	31·05	31·26
S . . .	0·55	0·38
P . . .	5·19	6·19
Fe . . .	0·29	...

[1] *Ztschr. f. physiol. Chem.*, Strassburg, Bde. xviii. and xx.

[2] "Weitere Beitr. z. Blutlehre," Wiesbaden, 1895.

[3] Details with reference to the influence of nucleo-proteids on blood coagulation are given in the article dealing with that subject.

[4] Halliburton and Brodie, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvii. p. 135.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1884, Bd. ix. S. 49.

If the iron is, as it appears to be, in organic union, the nucleins that contain it must be among the most complex of known organic compounds, consisting of seven elements.

The exact method in which the iron is combined is however, like the constitution of nuclein, still unknown.

Zaleski[1] has succeeded in separating from the liver one of these iron-containing nucleins, which he terms *hepatin*. The subject has been largely worked by microchemical methods for the detection of iron; and the terms "firmly combined" and "loosely combined" iron are often used, according as the compounds which contain that element give the reactions with difficulty or ease. Macallum[2] finds that the chromatin of nuclei contains iron; he regards it as the mother substance of hæmoglobin, both in embryological development and during nutrition in extra-uterine life. He finds similar hæmatogens in plants, as did also Bunge.

Lecith-albumins.—Liebermann[3] has given the name lecith-albumins to certain compounds of lecithin and proteid which he obtained from the kidney, gastric mucous membrane, lungs, spleen, and liver. The lecithin is not removable from these compounds by simple extraction with alcohol and ether. These, however, can hardly be considered to be immediate constituents of the cells, as they are obtained after subjecting them to a very severe process, namely, artificial gastric digestion. They yield no phosphoric acid and no xanthine bases on decomposition. According to their discoverer, they play an important part (in virtue of the acidity which they possess in common with nuclein compounds) in the separation of the hydrochloric acid of the gastric juice, and in decomposing the alkaline salts of the blood plasma, so as to yield the acid salts of the urine. Much more extended investigations are needed, however, before important functions like these can be safely attributed to them.

We have already seen that *vitellin* is a proteid which by some is regarded as a globulin, by others as a nucleo-proteid. Hoppe-Seyler[4] was inclined to regard the phosphorus found in it as due to a combination with lecithin, whereas Hammarsten looks upon some forms of vitellin as phospho-gluco-proteids. No doubt, vitellin is a name which covers a number of different substances; the substance Hoppe-Seyler worked with contained as much as 25 per cent. of lecithin. In those cases where the phosphorus is present as a nuclein, the nuclein obtained by gastric digestion is of the pseudo-nuclein variety.

The Albuminoids.

The albuminoids form a heterogeneous group of substances allied to the proteids, but differing from them by certain marked characteristics. As a rule, they are found in skeletal and epidermal structures, and usually they are remarkable for their resistance to reagents. They

[1] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. x. S. 453; xiv. S. 274; *Chem. Centr.-Bl.*, Leipzig, 1888, S. 759. See also Quincke, *Deutsches Arch. f. klin. Med.*, Leipzig, Bd. xxv. S. 567; xxvii. S. 202; xxxiii. S. 23; Peters, *ibid.*, Bd. xxxii. S. 182.

[2] Macallum's most recent papers are in *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 268; *Proc. Roy. Soc. London*, 1895, vol. lvii. p. 261; l. 277; *Quart. Journ. Micr. Sc.*, London, 1896, vol. xxxviii. p. 175; *Rep. Brit. Assoc. Adv. Sc.*, London, 1896.

[3] *Arch. f. d. ges. Physiol.*, Bonn, Bde. l. and liv.

[4] "Med. chem. Untersuch.," 1868; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 479.

include keratin, elastin, collagen, gelatin, reticulin, amyloid substance, and a group of materials called skeletins.

Collagen.—Collagen is the mother substance of gelatin. It is the material of which the white fibres of connective tissue are made, and is the principal constituent of which the organic substratum of bone is composed; it is there called ossein. In cartilage the material called chondrigen is collagen mixed with the mucinoid materials of the cartilaginous matrix. Collagen has also been obtained from the flesh of cephalopods.[1]

By boiling with water, especially if it is faintly acidified, collagen is converted into gelatin; and gelatin is reconverted into collagen by heating it to 130° C. Hence collagen is regarded as the anhydride of gelatin (Hofmeister);[2] the reaction may be represented by the equation—

$$\underset{\text{(gelatin)}}{C_{102}H_{151}N_{31}O_{29}} - H_2O = \underset{\text{(collagen)}}{C_{102}H_{149}N_{31}O_{28}}$$

The above formulæ, however, cannot be regarded as more than provisional, for we are as ignorant of the molecular constitution of the albuminoids as of the proteids. Schützenberger attributes the formula $C_{76}H_{124}N_{34}O_{29}$ to gelatin, and regards the sulphur described by other investigators as due to admixture with proteid impurities. Hammarsten,[3] on the other hand, regards the sulphur, of which there is 0·6 per cent., as an integral part of collagen and gelatin.

Collagen is insoluble in water, alcohol, salt solutions, and dilute acids, and alkalis. It swells with dilute acids. Its decomposition products are the same as those of gelatin.

Gelatin.—Gelatin is a colourless, amorphous, and translucent substance; it swells but does not dissolve in cold water; it readily dissolves in hot water, and on cooling the solution, if its concentration is greater than 1 per cent., it sets into a jelly. It contains a considerable amount of ash, the removal of which lessens its power of gelatinising.[4]

Gelatin is precipitated by saturating its solution with neutral salts, like magnesium sulphate and ammonium sulphate.[5] This is also true for gelatin which has been altered by the action of hot water so as to be no longer or only partially gelatinisable.

Gelatin is not precipitated by acetic acid, nor by acetic acid and ferrocyanide of potassium, nor by most of the heavy metallic salts that precipitate proteids. It gives a violet colour with copper sulphate and caustic potash; it gives Millon's reaction, but only a faint xanthoproteic reaction.[6]

It is precipitated by mercuric chloride, and also, as in the process of tanning, by tannic acid. Gelatin is levorotatory.[7]

Derivatives of gelatin.—The prolonged action (twenty-four hours) of boiling water, or the shorter action of water heated above the boiling point, destroys the gelatinising power of gelatin. Gelatin, in fact, undergoes hydrolysis, being converted into the so-called gelatin peptones. Similar substances are formed during digestion. Hofmeister distinguished

[1] Hoppe-Seyler, "Physiol. Chem.," S. 97.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ii. S. 315.

[3] "Physiol. Chem.," 3rd German edition, S. 46. Analyses of gelatin were made in addition to those quoted above by Mulder, *Ann. d. Chem.*, Leipzig, Bd. xlv.; Fremy, *Jahresb. d. Chem.*, 1854; and Paal, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxv. S. 1208.

[4] Nasse and Krüger, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xix. S. 29.

[5] Nasse, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xli. S. 504.

[6] Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 215; *Berl. klin. Wchnschr.*, 1885, No. 2.

[7] Hoppe-Seyler gives $(\alpha)_D = -130°$ at 30° C. Nasse and Krüger give $(\alpha)_D = -136°$ to $-167·5°$.

two of these substances, which he named semiglutin and hemicollin. Chittenden and Solley[1] distinguish between proto- and deuterogelatose, and true gelatin-peptone. Paal[2] has obtained similar substances by the use of hydrochloric acid. By the use of Raoult's method, he gives the molecular weight of gelatin as 878 to 960, and of gelatin-peptone as 352.

Strong reagents like sulphuric acid, on putrefaction, decompose gelatin with the formation of glycocine,[3] leucine, various fatty acids, glutaminic acid, carbon dioxide and ammonia. The absence of tyrosine should be noted. Schützenberger,[4] who has worked with gelatin by the same methods as he used with proteids, considers that gelatin, like proteid, is a compound of urea with certain amido-acids.

The importance of gelatin as a proteid-sparing food, though it will not replace proteid entirely in a diet, will be considered under "Nutrition."

Chondrin is the name given to the impure gelatin obtained from cartilage (which see).

Elastin.—Elastin is a material yielded by the yellow fibres of connective tissue. It offers great resistance to reagents, and may be prepared from the ligamentum nuchæ by extracting the finely divided tissue successively with reagents in which it is insoluble, and in which adherent fatty, collagenous, and proteid matters dissolve (boiling water, 1 per cent. potassium hydroxide, 5 per cent. hydrochloric acid, alcohol and ether). By this means a substance free from sulphur is obtained. Chittenden and Hart,[5] in some of their preparations, omitted the extraction with potash, and in these a small percentage of sulphur (0·3) was obtained; this may be due to proteid impurities, or it may be loosely combined in the elastin molecule. Schwartz[6] has also prepared a sulphur-containing elastin from the aorta.

The following table shows the results of elementary analyses in percentages:—

	Müller.[7]	Tilanus.[8]	Horbaczewski.[9]	Chittenden and Hart.	Schwartz.
C . .	55·09–55·7	54·9–55·65	54·32	54·24	53·95
H . .	7·11–7·67	7·25–7·41	6·99	7·27	7·03
N . .	15·71–16·52	17·52–17·74	16·75	16·7	16·67
O . .	20·7–21·15	19·5–20·33	21·94	21·69	21·97
S . .	...	...	...	0·3	0·38

Derivatives of elastin.—Elastin is gradually and slowly dissolved by

[1] *Journ. Physiol.*, Cambridge and London, vol. xii. p. 25.
[2] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxv.
[3] On the preparation and estimation of glycocine from gelatin, see C. S. Fischer, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xix. S. 164; and Gonnermann, *Arch. f. d. ges. Physiol.*, Bonn, Bd. lix. S. 42.
[4] *Compt. rend. Acad. d. sc.*, Paris, tome cii. p. 1296. See also Buchner and Curtius, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xix. S. 850.
[5] *Ztschr. f. Biol.*, München, Bd. xxv. S. 368; *Stud. Lab. Physiol. Chem.*, New Haven, vol. iii. p. 19.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii.
[7] *Ztschr. f. rat. Med.*, Leipzig, Dritte Reihe, Bd. x. pt. 2.
[8] Gorup-Besanez, "Physiol. Chem.," Aufl. 3, S. 148.
[9] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vi. S. 330.

pepsin or trypsin.[1] Horbaczewski named the two products of digestion he obtained, hemielastin and elastin-peptone. Chittenden and Hart, using Kühne's methods and nomenclature, have shown that hemielastin is protoelastose, and elastin-peptone is deuteroelastose.

On more complete decomposition elastin yields products very like those obtained from proteids, except that glycocine is obtained, but no aspartic or glutamic acid, and very little tyrosine.[2] Lysatinine but no lysine was obtained.[3] By fusing with potash, indol, skatol, phenol, benzene, but no methylmercaptan, were yielded (Schwartz).

Reticulin.—The fibres of reticular tissue, though histologically not distinguishable from those of areolar tissue, were first stated to be chemically different from them by Mall.[4] He asserted that no gelatin was obtainable from them, a statement corrected by R. A. Young,[5] and subsequently by Siegfried.[6] Siegfried, however, confirmed Mall's idea that the fibres contained something special, and separated from them a material he called reticulin. Reticulin has the following percentage composition:—C, 52·88; H, 6·97; N, 15·63; S, 1·88; P, 0·34; ash, 2·27. By decomposition it yields sulphuretted hydrogen, ammonia, lysine, lysatinine, and amidovalerianic acid, but no tyrosine and no glutaminic acid. It gives the proteid reactions with the exception of Millon's.

Siegfried prepared reticulin from the mucous membrane of the intestine by digestion with trypsin and alkali. The residue was washed and extracted with ether, again subjected to tryptic digestion, and extracted with alcohol and ether; the collagen was removed by hot water.

If glutaminic acid is absent, as Siegfried states, from the decomposition products of reticulin, and it is certainly very abundant in the decomposition products of collagen and gelatin, there is distinct evidence that reticulin is a new material.

We are therefore confronted with the difficulty, that the fibres of reticular tissue are anatomically continuous with and histologically identical with the white fibres of connective tissue, and yet they contain chemically this new material. The answer to the problem is probably that reticulin is not specially characteristic of reticular fibres, but is present in all white connective tissue fibres.

Keratin.—Keratin is the horny material of which the horny layer of the epidermis, hair, wool, nails, hoofs, horns, feathers, etc., are composed.

It is prepared by successively boiling the tissue with ether, alcohol, water, and dilute acid; the insoluble residue is keratin. A variety of keratin called *neurokeratin* is found in neuroglia, and has also been described in the medullary sheath of nerve fibres; though here no doubt some of the histological appearances described may be artificially produced by reagents. It resembles keratin in its general properties, but is less easily soluble in boiling solutions of caustic potash.[7]

[1] Kühne and Ewald, "Die Verdauung als histol. Methode," *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1877, N. F., Bd. i. S. 451; Etzinger, *Ztschr. f. Biol.*, München, Bd. x. S. 84; Horbaczewski, *loc. cit.*; Morochewetz, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1886, S. 271; Chittenden and Hart, *loc. cit.*

[2] Drechsel, Ladenburg's "Handwörterbuch," Bd. iii.; see also Horbaczewski, *Monatsh. d. Chem.*, Wien, Bd. vi.

[3] See, however, Hedin's recent work referred to on p. 33 of this article.

[4] *Anat. Anz.*, Jena, 1888, Bd. iii. No. 14; *Abhandl. d. math.-phys. Cl. d. k. sächs. Gesellsch. d. Wissensch.*, 1887, Bd. xiv. No. 3; xvii. No. 14.

[5] *Journ. Physiol.*, Cambridge and London, vol. xiii. p. 332.

[6] "Habilitationschrift," Leipzig, 1892.

[7] Ewald and Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, N. F., Bd. i. Heft 5; Kühne and Chittenden, *Ztschr. f. Biol.*, München, Bd. xxvi. S. 291.

The following are some elementary analyses that have been made of keratin from different sources:—

Tissue . .	From Hair.	Nail.	Neurokeratin.	Horn.
Analyst .	V. Laar.[1]	Mulder.[2]	Kühne.[3]	Horbaczewski.[4]
C . .	50·60	51·00	56·1–58·4	50·86
H . .	6·36	6·94	7·2–8·0	6·94
N . .	17·14	17·51	11·5–14·3	...
O . .	20·85	21·75	...	...
S . .	5·00	2·80	1·6–2·2	3·30

The main feature in the above analyses is the high percentage of sulphur,[5] which is in part in loose combination, and can be removed by alkalis or even by boiling water.

An albuminoid obtainable from tracheal cartilage by C. T. Mörner,[6] and further investigated by Hedenius,[7] is included by Hammarsten[8] among the keratins, or as a substance intermediate between keratin and coagulated proteid. It contains only 1 per cent. of sulphur. Keratin gives the proteid reactions.

Derivatives of keratin.—Keratin is not digestible by either gastric or pancreatic juice. By heating with water to 150°–200° C. it dissolves, forming a turbid solution. It dissolves more readily in alkalis; the solution contains alkaline sulphides, and substances of the proteose class, called keratinoses by Krukenberg.[9]

The decomposition products of keratin obtained by the use of acids are like those of the proteids, and include leucine, a good deal of tyrosine (1–5 per cent.), aspartic acid,[10] glutaminic acid,[11] ammonia, and sulphuretted hydrogen, lysine,[12] lysatinine,[12] and a sulphur-containing substance[12] which forms a compound with hydrochloric acid, with the formula $C_{14}H_{38}N_4O_{12}SCl_4$. Drechsel[13] considers that some of the oxygen of the keratin is united to sulphur, and a part to amido-acid radicles.

The close chemical relationship of keratin to proteid coincides with what is known as to its formation within the protoplasm of cells, for instance in the epidermis. The *eleidin* granules of the *stratum granulosum* probably represent an intermediate stage in the transformation.

[1] *Ann. d. Chem.*, Leipzig, Bd. xlv.

[2] "Versuch. einer allgem. physiol. Chem.," Braunschweig, 1844-51.

[3] Kühne and Chittenden, *loc. cit.*

[4] See Drechsel, Ladenburg's "Handwörterbuch," Bd. iii. Other analyses of horn have been made by Tilanus, Hoppe-Seyler's "Physiol. Chem.," S. 90; Lindvall, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1881.

[5] A large number of estimations of sulphur in keratins from different sources will be found in a paper by Mohr, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 403. The percentage varies from 2·6 to 5·3. Düring (*ibid.*, 1896, Bd. xxii. S. 281) obtained very similar results.

[6] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xviii. S. 217.

[7] *Skandin. Arch. f. Physiol.*, Leipzig, Bd. iii.

[8] "Physiol. Chem.," 3rd German edition, S. 44.

[9] *Sitzungsb. d. Jenaisch. Gesellsch. f. Med. u. Naturw.*, 1886.

[10] Kreusler, *Journ. f. prakt. Chem.*, Leipzig, Bd. cvii.

[11] Horbaczewski, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. lxxx.

[12] Hedin, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1893, Bd. xxii.

[13] *Loc. cit.*

Amyloid Substance.—This material, also called *lardacein*, occurs in disease in the form of degeneration, called waxy, albuminoid, amyloid, or lardaceous. It principally affects small blood vessels, but it may involve the tissue elements of organs. The degeneration occurs specially in cases of chronic pus formation, and is frequently a sequela of syphilis. The name amyloid was given to it, because the substance is coloured brownish red by iodine, and was supposed by Virchow to be of carbohydrate nature. Friedrich and Kekule[1] were the first to show that it is nitrogenous, and gave as its percentage composition, C, 53·6; H, 7; N, 15; O, 24·4. It also contains 1·3 per cent. of sulphur.[2] On decomposition it yields leucine, tyrosine, and the other products usually obtained from albuminous matter, but no sugar or other reducing substance. By boiling with alkali a chitin-like residue is left.[3] It is slowly soluble in gastric juice.[4]

Skeletins.—This term is applied by Krukenberg[5] to a number of nitrogenous substances found in the skeletal tissues of invertebrates. They are characterised by great insolubility, and are probably all amido-derivatives of carbohydrates. Under the term are included chitin, conchiolin, spongin, cornein, fibroin, and sericin.

Chitin.—This substance forms the chief constituent of the ectodermal skeletal tissues of invertebrate animals, especially of arthropods.[6] In crustacea it is often impregnated with calcareous matter, and in the odontophore of molluscs with silica. According to Krawkow,[7] it is in union with a proteid-like substance. Gibson,[8] Winterstein,[9] and Escombe[10] have found chitin instead of cellulose in several fungi.

It is prepared from the wing-cases of beetles by boiling them with caustic soda. The chitin remains insoluble; it may be dissolved in cold concentrated hydrochloric acid, and precipitated unchanged from this solution by the addition of water. It is colourless, amorphous, insoluble in water, alcohol, ether, acetic acid, dilute mineral acids, and concentrated solutions of the alkalis. It is soluble in concentrated mineral acids.

The formula and constitution of chitin are differently given by different observers. Ledderhose[11] gave it the formula $C_{15}H_{26}N_2O_{10}$. Berthelot[12] stated that it yields a fermentable sugar on boiling with sulphuric acid. Sundwik[13] gave it the formula $C_{60}H_{100}N_8O_{38} + nH_2O$ (n varying from 1 to 4), and considered it to be an amine derivative of a carbohydrate with the formula $(C_{12}H_{20}O_{10})_n$. Krawkow[14] considers

[1] *Virchow's Archiv*, Bd. xvi. S. 58. [2] Kühne and Rudneff, *ibid.*, Bd. xxxiii.

[3] Krawkow, *Centralbl. f. d. med. Wissensch.*, Berlin, 1892. The resemblance to chitin is supported only by its behaviour to staining agents like iodine. There is no true chemical resemblance between the two substances. Amyloid substance, for instance, yields no glucosamine (Cohn, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 153).

[4] Kostiurin, *Wien. med. Jahrb.*, 1886, S. 181. See also Tschermak, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 343.

[5] *Ztschr. f. Biol.*, München, Bd. xxii. S. 241; "Grundzüge einer vergl. Physiol. d. thier. Gerüstsubstanz," Heidelberg, 1885.

[6] Gamgee ("Physiol. Chem.," vol. i. p. 299) gives a list of the situations where chitin has been described or inferred to exist. To these must be added the pen of cuttle-fishes (Krukenberg, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xviii. S. 989); and the cartilages and other mesoblastic structures of the sepia and king crab (Halliburton, *Proc. Roy. Soc. London*, vol. xxxviii. p. 75).

[7] *Ztschr. f. Biol.*, München, Bd. xxix. [8] *Compt. rend. Acad. d. sc.*, Paris, tome cxx.

[9] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1894 and 1895.

[10] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 288.

[11] *Ibid.*, Bd. ii. S. 213; iv. S. 139.

[12] *Compt. rend. Acad. d. sc.*, Paris, tome xlvii. p. 227.

[13] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. v. [14] *Loc. cit.*

that there are a number of chitins, amine derivatives of different carbohydrates (dextrose, glycogen, dextrin, etc.); they give different colour reactions with iodine.[1] Ledderhose[2] was the first to show that the reducing substance obtained by the action of mineral acids in chitin is not sugar but glucosamine (see p. 9). The equation representing its decomposition he gives as follows:—

$$\underset{\text{(chitin)}}{2C_{15}H_{26}N_2O_{10}} + \underset{\text{(water)}}{6H_2O} = \underset{\text{(glucosamine)}}{4C_6H_{13}NO_5} + \underset{\text{(acetic acid)}}{3C_2H_4O_2}$$

Glucosamine is an amido-derivative of glucose; it forms crystalline salts, of which the hydrochloride is readily prepared by boiling chitin with hydrochloric acid; this is soluble in water, and is dextrorotatory $(\alpha)_D = +70°{\cdot}6$. The base is prepared by the action of baryta on the sulphate. It is crystalline and not fermentable with yeast.

Schmiedeberg[3] looks upon chitin as an acetyl derivate of glucosamine, and as he has also obtained the latter substance from the chondroitin-sulphuric acid of cartilage, he regards it as indicating a connection between the skeletal tissues of vertebrate and invertebrate animals.

By heating chitin with ten times its weight of caustic alkali at 180°, Hoppe-Seyler and Araki[4] obtained a substance which possesses the original form of the pieces of chitin, but differs from chitin in being very soluble in dilute acids such as acetic acid; from such solutions it is precipitable by alkalis. This substance is called chitosan, and its formation from chitin is shown in the following equation:—

$$\underset{\text{(chitin)}}{C_{18}H_{30}N_2O_{12}} + \underset{\text{(water)}}{2H_2O} = \underset{\text{(chitosan)}}{C_{14}H_{26}N_2O_{10}} + \underset{\text{(acetic acid)}}{2C_2H_4O_2}$$

Chitosan in dilute acetic acid is levorotatory; $(\alpha)_D = -17°{\cdot}7$ to $17°{\cdot}9$. By heating it with acetic acid in sealed tubes to 135°, a substance very like chitin is regenerated; it, however, contains three, whereas true chitin only contains two acetyl groups.

By boiling with concentrated hydrochloric acid, chitosan yields hydrochloride of glucosamine, formic and acetic acids.

Neurochitin.—In crustacea, chitin has been said to take the place of neurokeratin as a support to the nerve fibres.[5]

Conchiolin ($C_{30}H_{48}N_9O_{11}$) forms the organic basis of the shells of mussels and snails. On decomposition it yields leucine, perhaps glycocine, but no tyrosine or reducing substance. It does not give the xanthoproteic, Millon's, nor the Adamkiewicz reactions. The byssus of molluscs is similar. The cementing substance between the eggs of various molluscs contains a substance more like keratin. *Cornein*, from corals ($C_{30}H_{44}N_9O_{33}$), differs from conchiolin by giving a red colour with Millon's test; on decomposition it yields leucine and a crystalline material called cornicrystallin.

Spongin, the organic basis of the common sponge, yields as decomposition products, leucine and glycocine (Städeler), but no tyrosine.[6] It does not give the colour reactions just mentioned; it resembles conchiolin by yielding

[1] E. Zander (*Arch. f. d. ges. Physiol.*, Bonn, 1897, Bd. lxvi. S. 545) also finds that chitin gives a colour with iodine very like that given by glycogen.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ii. S. 213; iv. S. 137.

[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxviii.

[4] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1894, Bd. xxvii. S. 3329; 1895, Bd. xxviii. S. 82; *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 498.

[5] Griffiths, *Compt. rend. Acad. d. sc.*, Paris, tome cxv.

[6] Zalocostas (*ibid.*, tome cvii. p. 252), however, obtained tyrosine, butalanine, and glucalanine ($C_5H_{12}N_2O_4$).

peptone-like materials on digestion, which differ from true peptones and proteoses by not giving the colour reactions in question.

Fibroin is the substance of which spiders' webs are composed. It is insoluble except in concentrated mineral acids and alkalis. It yields on decomposition glycocine, leucine, and tyrosine, and gives the proteid colour reactions. This substance and *sericin*, a similar material (which, however, gives no glycocine on decomposition), are found together in silk.[1] Hammarsten[2] gives the following table of percentage compositions :—

	C	H	N	S	O	
Conchiolin .	50·92	6·88	17·86	0·31	24·34	Krukenberg.[3]
Spongin .	46·5	6·3	16·2	0·5	27·5	Crookewitt.[4]
,, .	48·75	6·35	16·4	...	...	Possell.[5]
Cornein .	48·96	5·9	16·81	...	28·33	Krukenberg.
Fibroin .	48·23	6·27	18·31	...	27·19	Cramer.[6]
,, .	48·3	6·5	19·20	...	26·0	Vignon.[7]
Sericin .	44·32	6·18	18·30	...	30·2	Cramer.

Inorganic Compounds.

Water forms about 58·5 per cent. of the weight of the body; in infants it is 66·4 per cent. An adult takes in food 2,500 c.c. of water daily, and excretes rather more, as some is formed in the body by the oxidation of hydrogen.

Hydrogen peroxide is stated by Würster[8] to be given off in various situations; he uses tetramethyl-paraphenylenediamine papers to detect it.

Hydrogen sulphide occurs in small quantities as the result of putrefactive changes in the alimentary canal.

Ammonia is also formed in putrefactive processes, and in pancreatic digestion. A small quantity occurs in fresh urine, and increases when the urine putrefies.

Hydrochloric acid occurs in gastric juice.

Carbonic acid occurs in the blood, lymph, and secretions.

The acids found in the body are, however, usually in combination as salts.

Salts.—The chief salts found are the chlorides of sodium and potassium, the sulphates of the same metals, phosphates of sodium, potassium, calcium, and magnesium, carbonates of sodium and calcium. Bone, dentine, and enamel are chiefly rich in calcium salts, especially the phosphate. Other solid tissues are especially rich in potassium salts. In the fluids (milk excepted) the most abundant salt is sodium chloride.

A fuller consideration of the various saline constituents will be taken with the individual tissues and secretions. The following general tables may be, however, quoted here;[9] the figures give percentage quantities of mineral matters in the ash :—

[1] Weyl, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxi. S. 1407, 1529.
[2] "Physiol. Chem.," 3rd German edition, S. 49.
[3] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xvii.
[4] *Ann. d. Chem.*, Leipzig, Bd. xlviii.
[5] *Ibid.*, Bd. xlv.
[6] *Journ. f. prakt. Chem.*, Leipzig, Bd. xcvi.
[7] *Compt. rend. Acad. d. sc.*, Paris, tome cxv.
[8] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xix. S. 3195 ; xx. S. 263, 1033.
[9] From Beaunis, "Physiologie humaine."

Tissue . . .	Bone.	Calf-muscles.	Brain.	Liver.	Lungs.	Spleen.
Analyst . .	Heintz.	Staffel.	Breed.	Oidtmann	C. Schmidt.	Oidtmann.
Sodium chloride .	...	10·59	4·74	...	13·0	...
Soda . . .	...	2·35	10·69	14·51	19·5	44·33
Potash . .	...	34·40	34·42	25·23	1·3	9·60
Lime . . .	37·58	1·99	0·72	3·61	1·9	7·48
Magnesia . .	1·22	1·45	1·23	0·20	1·9	0·49
Ferric oxide .	...	...	...	2·74	3·2	7·28
Chlorine . .	...	...	...	2·58	...	0·54
Fluorine . .	1·66	...	...	...	...	...
Phosphoric acid .	53·31	48·13	48·17	50·18	48·5	27·10
Sulphuric acid .	...	...	0·75	0·92	1·4	2·54
Carbonic acid .	5·47	...	...	...	...	...
Silicic acid . .	...	0·81	0·12	0·27	...	0·17

Fluid . . .	Blood.	Serum.	Blood-clot.	Lymph.	Urine.	Milk.	Bile.	Excrements.
Analyst . .	Verdeil.	Weber.	Weber.	Dahnhardt.	Porter.	Wildenstein.	Rose.	Porter.
Sodium chloride	58·81	72·88	17·36	74·48	67·26	10·73	27·70	4·33
Potassium ,,	...	...	29·87	...	...	26·33	...	...
Soda . . .	4·15	12·93	3·55	10·35	1·33	...	36·73	5·07
Potash . .	11·97	2·95	22·36	3·25	13·64	21·44	4·80	6·10
Lime . . .	1·76	2·28	2·58	0·97	1·15	18·78	1·43	26·40
Magnesia . .	1·12	0·27	0·53	0·26	1·34	0·87	0·53	10·54
Ferric oxide .	8·37	0·26	10·43	0·05	...	0·10	0·23	2·50
Phosphoric acid .	10·23	1·73	10·64	1·09	11·21	19·00	10·45	36·03
Sulphuric acid .	1·67	2·10	0·09	...	...	2·64	6·39	...
Carbonic acid .	1·19	4·40	2·17	8·20	...	...	11·26	...
Silicic acid . .	...	0·20	0·42	0·42	4·06	...	0·36	3·13

Sodium and potassium salts. — Probably 200 grms. may be taken as an average amount of sodium chloride (common salt) in the adult human body. It is a most important food, and about 16 grms. are daily excreted in the urine,[1] and smaller amounts in the sweat and fæces. If potassium chloride be substituted in the food for the sodium salt, disturbances arise from deficiency of the latter. The tissues, however, retain common salt very tenaciously, so that during a dietary devoid of salt it disappears slowly from the urine.

During its passage through the body, it facilitates the absorption of proteid food, and increases tissue metabolism. The following table[2] gives the probable relative amounts of sodium and potassium chlorides in parts per thousand:—

	NaCl	KCl		NaCl	KCl
Blood . .	2·70	2·05	Pancreatic juice (from temporary fistula) .	7·35	0·02
Blood corpuscles	...	3·67	Gastric juice .	1·45	0·55
Plasma . .	5·54	0·35	Bile . . .	5·33	0·28
Lymph . .	5·67	...	Milk . . .	0·87	2·13
Chyle . .	5·84	...	Urine . .	11·00	4·50
Pancreatic juice (from permanent fistula) .	2·50	0·93			

Bunge found that the soda salts are more abundant in embryonic

[1] Vierordt's "Daten u. Tabellen," 1893, Aufl. 2, S. 122.
[2] From M'Kendrick's "Text-book of Physiology," Glasgow, 1888, vol. i. p. 39.

and early life than in adult life. This is illustrated by the following table:—

	Na_2O	K_2O		Na_2O	K_2O
Rabbit's embryo.	2·183	2·605	Cat 29 days old	2·292	2·684
Rabbit 14 days old	1·630	2·967	Dog 4 „ „	2·589	2·667
Kitten 1 day „	2·666	2·691	Adult mouse . .	1·700	3·280
Cat 19 days „	2·285	2·790			

This fact is probably due to the larger amount of cartilage (rich in soda salts) and the smaller amount of muscle (rich in potash salts) in early life as compared with the adult condition.[1]

Various phosphates of sodium and potassium are found in the blood, lymph, urine, and other secretions.

Sodium carbonate and bicarbonate occur in the food, and originate in the body from the salts of vegetable acids (tartaric, citric, etc.).

Sodium and potassium sulphate exist in smaller quantities in the body. Only minute quantities of these salts are introduced with the food; they are chiefly formed by the oxidation of proteids and other organic substances containing sulphur.

Ammonium salts.—Minute traces of ammonium chloride are found in the urine. The urine of reptiles and birds is largely composed of ammonium urate. Small quantities of this salt, and also of ammonio-magnesic phosphate, are found in human urine. Ammonium carbonate is formed from urea in decomposing urine.

Calcium salts.—About three-quarters of the total mineral solids in the body consist of calcium phosphate, $Ca_3(PO_4)_2$; this is because of the great preponderance of this salt in bone. Other calcium salts occurring in bone, dentine, and enamel are the carbonate, sulphate, and fluoride. Calcium phosphate, urate, and oxalate, are found in the urine. Most tissues contain small quantities of the phosphate and carbonate. Egg shells, the shells of crustacea, coral, and otoliths consist chiefly of carbonate of lime.[2]

Magnesium salts.—Magnesium phosphate ($Mg_3(PO_4)_2$) occurs in the tissues along with the calcium phosphates ($Ca_3(PO_4)_2$ and $CaH_4(PO_4)_2$) but in smaller amount. It occurs also in the urine. Ammonio-magnesium or triple phosphate ($NH_4MgPO_4+6H_2O$) is also often found in decomposing urine. Magnesium palmitate and stearate are found in the fæces.

Iron is an important constituent of the blood pigment. The blood of an adult contains 3 grms. of iron. Small quantities are found in other liquids of the body (chyle, lymph, bile, milk, urine, gastric juice); it is also contained in the black pigment of the skin and hair, and of melanotic sarcomata. A small quantity of ferric sulphide is found in the fæces, and small quantities of iron are found in both liver and spleen.[3] It is present in the tissues in organic combination with nuclein (see p. 68).

Copper is found in two proximate principles, hæmocyanin, the blue pigment of the blood of many invertebrates (crustacea, cuttle-fishes, scorpions, etc.), and in the pigment, turacin, of birds' feathers. Small quantities of this metal, and also of aluminium, manganese and lead, may occur accidentally in

[1] Bunge, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 399.

[2] On excretion and absorption of lime see Rey, *Chem. Centr.-Bl.*, Leipzig, 1895, Bd. ii. S. 55, 837.

[3] For data concerning the amount of iron in foods, see Stockman, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 484; 1897, vol. xxi. p. 55. An ordinary daily diet contains 9–10 mgrms. of iron.

other parts, being taken in with the food,[1] and not excreted at once with the fæces, but deposited in some tissue or organ. Drugs and poisons (mercury, arsenic) may be similarly deposited.

Silica.—A minute quantity of silica exists in the blood, urine, bones, hair, and other parts.

Phosphates.—The amount of phosphoric acid given in analyses of the ash of animal structures is not always correct, since a certain quantity is obtained during the process of incineration, from the decomposition of organic compounds, which, like lecithin, contain phosphorus.

The phosphoric acid which occurs in mineral compounds in the body is derived in part directly from the food, and in part from the metabolism of lecithin and nuclein. It unites with soda, potash, lime, and magnesia to form the various phosphates already alluded to. An adult man eliminates by the kidneys 2·5 to 3·5 grms. of phosphoric acid daily. Carnivora eliminate phosphates chiefly by the kidneys, herbivora chiefly with the fæces.

Carbonates.—The presence of carbonates in the ash of animal matters is partly derived from the decomposition of organic compounds. Alkaline carbonates and bicarbonates are, however, found in blood, urine, lymph, saliva, etc.

Sulphates.—These also may be partly formed during the process of incineration, from proteids and other organic compounds containing sulphur. The sulphuric acid in the urine is in part combined as ordinary sulphates, in part as ethereal sulphates. It is derived to a small extent from the food, but chiefly from the metabolism of proteids, the amounts of sulphuric acid and urea in the urine running parallel.

[1] Karl B. Lehmann (*Arch. f. Hyg.*, München u. Leipzig, Bd. xxiv. S. 1, 18, 72) states that in an ordinary diet we take 20 mgrms. of copper daily, and if preserves are much used, it may rise to over 300 mgrms. per diem ; more than 120 mgrms. appears to be harmful.

THE CHEMISTRY OF THE TISSUES AND ORGANS.

By W. D. Halliburton.

Contents.—Cells and Protoplasm, p. 80; Liver, p. 85; Spleen, p. 87; Thymus, p. 88; Thyroid, p. 88; Suprarenals, p. 90; Pancreas, p. 92; Kidneys, p. 92; Testis, p. 92; Muscle, p. 95; Skeletal Tissues, p. 111; Nervous Tissues, p. 115; The Eye, p. 121; Milk, p. 125.

The preceding article contains an account of the principal proximate principles occurring in the body and in food.

In the present article I propose to present the subject from another standpoint, and to discuss the chemical composition of the various animal tissues and organs. This will in great measure be complemental to what has been already done, and will give the opportunity of describing some substances which have only been treated incidentally in the foregoing chapter.

In describing the chemistry of the organs, I shall endeavour to avoid discussions as to their metabolic functions, and shall omit all consideration of their secretions, since these are treated elsewhere in this work; an exception, however, will be made in the case of milk.

Protoplasm and cells.—The chemical structure of living substance is still beyond our knowledge. All that chemists are able to do is to examine the disintegration products of the substance which they unavoidably kill by the use of reagents.

Some authors speak of living substance as if it were merely proteid in composition, and have adopted the phrase "living proteid" (see p. 38). But it is doubtful if the use of such a term is justifiable, for protoplasm even in its simplest condition invariably contains, or yields on disintegration, substances other than proteid, though proteids and compound proteids like nucleo-proteid are by far the most abundant of these disintegration products. Among the other solid substances constantly present in protoplasm are lecithin, cholesterin, and inorganic salts (especially phosphates and chlorides of calcium, sodium, and potassium); and frequently fat and carbohydrate material, such as glycogen, are also to be found. Water occurs to the extent of 75 per cent. or more. Whether these substances are all present in the free state, or, as is much more probable, are linked together in intimate union, to form the complex protoplasmic molecule, it is at present impossible to say with certainty. Living cells are alkaline; after death they become acid.

The simplest form of protoplasm known is that found in the plasmodium of the myxomycetous fungus, *Æthalium septicum*. It has

been analysed by Reinke[1] and Krukenberg,[2] and their observations confirm what has just been stated.

The nucleus of cells, the study of which began with the work of Brunton, Plósz, and Miescher, has of recent years been very thoroughly worked at by Hoppe-Seyler, Kossel, and numerous other physiological chemists; the result will be gathered from the section in the preceding chapter on nuclein, and, as will be there seen, there are yet many gaps in our knowledge which require to be filled up.

The proteids obtained from the cell protoplasm have been examined in simple cells such as those of lymphoid tissue, and in the more specialised cells of secreting organs, such as the liver, kidneys, testis, and so forth. The main result is the same in all, though there are minor differences between individual cases.

The proteid contained in greatest abundance is nucleo-proteid; small quantities of globulin usually coagulating at the low temperature of 50° C. or even lower, and minute traces of an albumin are also found.

The nucleo-proteids from different cells differ in the amount of nuclein (as evidenced by the percentage of phosphorus) they contain.[3]

The nucleo-proteid from the	thymus	contains	0·8	per cent. of	phosphorus.
,, ,,	kidney	,,	0·37	,,	,,
,, ,,	liver	,,	1·45	,,	,,
,, ,,	brain	,,	0·5	,,	,,
,, ,,	red marrow	,,	1·6	,,	,,
,, ,,	red corpuscles	,,	0·68	,,	,,
Schmidt's fibrin ferment		,,	1·25	,,	,,

In my early work[4] on the proteids of cell protoplasm, I selected the cells of lymphatic glands, because one can obtain from these structures an abundant supply of comparatively simple cells; later, I found that the cells of thymus[5] gave similar results. At first I described the proteids obtained as four in number, namely nucleo-proteid, cell globulin-α, cell globulin-β, and cell albumin. The nucleo-proteid can be obtained either by Wooldridge's acetic acid method or by the sodium chloride process (p. 68).

The material obtained by both methods is the same, though they differ in their physical condition; that obtained by the sodium chloride process being more viscous than that by Wooldridge's method. That they are the same is shown by the facts that both give the same reactions, which closely resemble those of globulins; both contain practically the same amount of phosphorus, and both produce intravascular coagulation.[6]

The term *cell globulin* was originally introduced by me as a convenient designation for the proteids which are coagulable by heat in sodium sulphate extracts of the cells. The nucleo-proteid just mentioned is viscid when extracted by sodium chloride and magnesium sulphate, but an extract with sodium sulphate solution does not exhibit

[1] "Studien ueber das Protoplasma," Berlin, 1881.
[2] *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1882, Bd. ii. S. 273.
[3] Halliburton, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 306.
[4] *Proc. Roy. Soc. London*, 1888, vol. xliv. p. 255; *Journ. Physiol.*, Cambridge and London, 1888, vol. ix. p. 229.
[5] *Ibid.*, vol. xviii. p. 306.
[6] Halliburton and Brodie, *ibid.*, 1894, vol. xvii. p. 135.

viscidity, and it was the absence of this character which led me to the erroneous conclusion that no nucleo-proteid had gone into solution.

The sodium sulphate extract contains two proteids, one which coagulates at 48°–50° C., the other at 75° C. The first, which I called cell globulin-α, is really a globulin; it yields no nuclein on gastric digestion;[1] but the second, which I called cell globulin-β, though like a globulin in its solubilities, is really the same nucleo-proteid which by treatment with other salts is rendered viscid.[2] That this substance is related to, if not identical with, the fibrin ferment or its zymogen (Pekelharing) has been rendered probable by the researches of Pekelharing and myself.

The *albumin* is only present in minute quantities; its properties are like those of serum albumin, and it may partly arise from blood or lymph imperfectly washed away from the cells.

Proteoses and peptone, when present, are the result of post-mortem changes, or of manipulations during the processes employed in separating the other proteids.

Myosin is absent.

Lilienfeld[3] has carried out a similar research on the chemistry of cells which he obtained from the thymus, by the usual means of pressure and the centrifuge. He found a proteid corresponding to cell globulin-α coagulating at 48° C., and another corresponding to cell albumin coagulating at 73–75° C. The nucleo-proteid which he obtained by my sodium chloride process contained C, 53·46; H, 7·64; N, 15·57, and P, 0·433 per cent. The alcoholic extract of the cells contained protagon, amido-valeric acid, inosite, and monopotassium phosphate.

By Wooldridge's method he obtained the nucleo-proteid he has called "nucleo-histon" (see p. 68), and he considers that this, in part at any rate, is derived from the nuclei. Its percentage composition is C, 48·46; H, 7·0; N, 16·86; P, 3·025; and S, 0·701. The action of artificial gastric juice, or of 0·8 per cent. hydrochloric acid, on this, is to separate the nuclein from the proteid, which goes into solution as peptone. The nuclein contains 4·991 per cent., and the nucleic acid prepared from this 9·94 per cent., of phosphorus.

In the following table he gives the quantitative composition of leucocytes:—

Water	88·51
Solids	11·49

One hundred parts of the solids contain—

Total phosphorus	3·01
Total nitrogen	15·03
Proteid	1·76
Nuclein	68·78
Histon (*i.e.* proteid part of the nucleo-proteid)	8·67
Lecithin	7·51
Fat	4·02
Cholesterin	4·40
Glycogen	0·80

[1] Halliburton, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 806.
[2] *Ibid.*, 1895, vol. xviii. p. 312. Pekelharing showed this also to be the case.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 473.

The high percentage of phosphorus in the nucleo-proteid obtained by this method is certainly not in accord with the observations of Brodie and myself.

We are justified in concluding from this work that the colourless corpuscles of the blood which originate from lymphoid structures have a similar composition. It is, however, impossible to investigate the actual colourless blood corpuscles by macrochemical methods. Microchemically they can be shown sometimes to contain fat and glycogen.[1]

Pus cells are colourless corpuscles, which show a considerable amount of fatty degeneration and are generally dead; these have been the subject of several researches. The nuclei consist of nuclein, which is historically interesting, because this was the first preparation made by the method of gastric digestion (Miescher).[2]

The protoplasm consists of proteids chiefly, but it also yields extractives and inorganic salts. Hoppe-Seyler's analysis of two samples of dried pus cells give the following percentage results :—

	I.		II.
Proteids	13·762		
Nuclein	34·257	68·585	67·369
Insoluble substances	20·566		
Lecithin	14·383		7·564
Fats			7·500
Cholesterin	7·400		7·283
Cerebrin	5·199		10·284
Extractives	4·433		

Inorganic constituents in one hundred parts of dried pus corpuscles—

NaCl	0·435	PO_4	0·916
$Ca_3(PO_4)_2$	0·205	Na	0·068
$Mg_3(PO_4)_2$	0·113	K	traces.
$Fe_2(PO_4)_2$	0·106		

Proteids of pus.—Boedecker[3] asserted that pus occasionally contains gelatin and chondrin in addition to proteids, and a crystalline acid he termed chlorrhodinic acid, but Miescher was unable to confirm these results; Miescher was also unable to find any myosin, a substance previously supposed to exist in the cell protoplasm.

My own observations coincide with those of Miescher on this point, and also show that the most abundant proteid is nucleo-proteid. In fact, the proteids obtained from pus are practically the same as those from the thymus and other lymphoid structures.

Fibrin ferment was prepared from pus by Rauschenbach.[4] Considerable quantities of proteoses and peptone are generally found in pus, and are doubtless produced during the retrogressive metamorphosis of the corpuscles. The original statement that pus contains peptone was made by Eichwald[5] and Hofmeister.[6] Though the method they employed was not perfectly trustworthy, S. Martin[7] showed that they were right in their conclusions. He placed pus under alcohol for many weeks,

[1] Schäfer, "Course of Practical Histology," London, 1876, p. 39.
[2] Hoppe-Seyler's "Med. Chem. Untersuch.," 1871, Heft 4, S. 497.
[3] *Ztschr. f. rat. Med.*, Leipzig, N.F., Bd. vi.
[4] Inaug. Diss., Dorpat, 1883; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden Bd. xiii. S. 134.
[5] *Verhandl. d. phys-med. Gesellsch. zu Würzburg*, 1864, S. 335.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, 1864, Bd. ii. S. 295.
[7] *Brit. Med. Journ.*, London, 1890, vol. ii. p. 234.

and then dried it and extracted it with water. Proteoses and peptone went into solution, the other proteids having been coagulated. It is probable that some of the symptoms which accompany the suppurative process is produced by the entrance of these substances into the circulation.[1]

There is little to add concerning the other constituents of pus cells. The large increase of fat, lecithin, and cholesterin confirms the fact of fatty degeneration, evident to the microscope; free fatty acids may even be found in old pus, forming crystalline deposits.

Glycogen can often be demonstrated in pus corpuscles microchemically by the use of iodine;[2] Salomon[3] separated it from the cells in appreciable quantities.

Pigments (pyocyanin, pyoxanthose) are frequently found in pus, and are produced by chromogenic bacteria (Fordos, Lücke, Fitz, Kunz, Babes).[4]

The proteids of red marrow cells.—In the lymphatic glands and thymus the cells are non-eosinophile; in the red marrow the cells are mostly eosinophile. Sherrington[5] showed that the eosinophile granules give microchemically the reaction for phosphorus, introduced by Lilienfeld and Monti;[6] the cells themselves were investigated macrochemically by J. R. Forrest.[7] The marrow used was obtained from the interior of rabbits' femora and horses' ribs.

His results were very like those obtained from other cellular structures. Two proteids only were obtainable in any quantity, these were a cell globulin, coagulating at 47°–50°, and a nucleo-proteid. The latter contains a high percentage of phosphorus,[8] namely, 1·6. Hæmoglobin is present in small quantities, and proteose and peptone are absent.

Epithelium.—Our knowledge of the tissues included under the heading epithelium is principally histological. There is no reason to suppose that the proteid constituents of the protoplasm and nucleus are in any way different from that found in cells generally.

Mucin is formed in many situations, both in the cells of mucous glands and in goblet cells. It is also the principal constituent of the cementing material between the cells.

Mucus is the name given to the secretion which owes its sliminess to mucin. Mucus also contains epithelial cells, more or less disintegrated, and a few leucocytes. It has an alkaline reaction, and contains a certain small proportion of proteids, extractives, and salts, similar to those of the blood. In some cases, the mucinoid material in secretions is really a nucleo-proteid. Thus, in the bile of some animals like the ox, there is very little true mucin, but the viscidity is almost entirely due to nucleo-proteid;[9] in human bile, on the other hand, the viscid material is mucin, very little nucleo-proteid being

[1] Ott and Collmar, *Journ. Physiol.*, Cambridge and London, vol. viii. p. 218; Krehl and Matthes, *Deutsches Arch. f. klin. Med.*, Leipzig, 1896, Bd. liv. S. 501; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, Bd. xxxvi. S. 437.

[2] Ranvier, *Progrès méd.*, Paris, 1877, p. 422.

[3] *Deutsche. med. Wchnschr.*, Leipzig, 1877, No. 35.

[4] *Compt. rend. Acad. d. sc.*, Paris, 1860, tome li. p. 215; *Arch. f. klin. Chir.*, Berlin, 1862, Bd. iii. S. 125; *Quart. Journ. Micr. Sc.*, London, Jan. 1880, p. 106; *Monatsh. d. Chem.*, Wien, Bd. ix. S. 361; *Compt. rend. Soc. de biol.*, Paris, 1889, p. 438.

[5] *Proc. Roy. Soc. London*, 1894, Bd. lv. p. 161.

[6] *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xvii. S. 410.

[7] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvii. p. 174.

[8] Halliburton, *ibid.*, 1895, vol. xviii. p. 307.

[9] Paijkull, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 196.

present.[1] The mucus of urine has also been stated to be nucleo-proteid in nature.[2] K. A. H. Mörner[3] investigated healthy human urine; each experiment necessitated the use of 80–90 litres. He found proteid or proteid-like materials partly in suspension in the ordinary mucous cloud or nubecula, and partly in solution. From the nubecula he separated a specific member of the mucin group, which he calls urine mucoid. This probably originates from the mucous membrane of the urinary passages. It contains C 49·4, N 12·74, and S 2·3 per cent.; and in its general properties agrees with ovo-mucoid pretty closely (see p. 63). The soluble proteid in urine, which is present in the merest traces, is chiefly serum albumin, but some is precipitable by acetic acid, and this part consists of a nucleo-proteid; precipitated with it was found a small quantity of chondroitin sulphuric acid (see "Cartilage").

The mucin of the respiratory passages has been investigated by F. Müller.[4] He finds it is true mucin, not nucleo-proteid. It yields from 25 to 32 per cent. of reducing substance. This is a nitrogenous derivative of a hexose, and is probably glucosamine.

Keratin and the *skeletins* are epithelial products which have already been described (p. 72). The enamel of teeth, although epithelial in origin, will be taken with the skeletal tissues. The epithelium of secreting glands will be studied with those glands and their secretions.

Glandular Organs.

The liver.—The fresh liver is alkaline in reaction, but after death soon becomes acid from the development of sarcolactic acid.

The number of organic substances in the liver is very numerous. There are proteids and nuclein from the hepatic cells; there are substances like glycogen, sugar, and fat, stored up within the cells, or produced from stored-up substances. Gelatin and mucin are obtainable from the connective tissue framework. There are also extractives like xanthine, hypoxanthine, and uric acid; lastly, a small proportion of inorganic constituents.

The proportion of water is about 75 per cent. v. Bibra[5] gives the following numbers:—

Water . .	76·17 per cent.	Gelatin . .	3·37 per cent.
Insoluble tissues	9·44 „	Extractives .	2·40 „
Proteids . .	2·40 „	Fats . .	2·50 „

Proteids of the liver cells.—These were first investigated by P. Plósz.[6] He found that, accompanying the onset of acidity after death, the liver became harder and less transparent; he therefore compared the condition to the *rigor mortis* of muscle, and sought for myosin by the methods Kühne had introduced for separating muscle plasma. He did not, however, find any myosin. He extracted the proteids by means of saline solutions of various strengths, and found—

(1) A proteid coagulating at 45° C., wholly soluble in gastric digestion; (2) a nucleo-proteid, coagulating at 70° C., yielding an insoluble residue of

[1] Hammarsten, *Kon. Ges. der Wiss.*, Upsala, 1893 (*Separat-abzug*); Baginsky and Somerfeld, *Verhandl. d. physiol. Gesellsch.*, Berlin, 1894–5, Nos 13, 14, 15 in *Arch. f. Physiol.*, Leipzig, 1895, S. 362.

[2] Lönnberg, *Upsala Läkaref. Förh.*, vol. xxv.; K. Mörner, *Hygiea*, Stockholm, 1892, vol. lii; Obermayer, *Centralbl. f. klin. Med.*, Bonn, Bd. xii.

[3] *Skandin. Arch. f. Physiol.*, Leipzig, 1895, S. 332.

[4] *Sitzungsb. d. Gesellsch. z. Beförd. d. ges. Naturw. zu Marburg*, 1896, No. 6.

[5] v. Bibra, "Chemische Fragmente ueber die Leber," 1849.

[6] *Arch. f. d. ges. Physiol.*, Bonn, Bd. vii. S. 371.

nuclein on gastric digestion; (3) a globulin coagulating at 75° C.; and (4) alkali albumin.

A good many years later,[1] I repeated these experiments; and, like Plósz, failed to find myosin. Myosin appears to be a specific constituent of muscle, and has not been found anywhere else. The hardening that occurs in the liver after death, and which is very slight, is possibly due to the solidification of the fat in the cells; though it is also quite possible, as Plósz suggests, that if coagulation does occur in the cells with the formation of a myosin-like clot, this takes place so rapidly that our present methods do not enable us to separate its precursor from the cells.

The proteids I obtained by the use of saline solutions were four in number:—

1. A globulin (cell-globulin) coagulating at 45°–50° C.
2. A nucleo-proteid which coagulates at about 60° C., and is identical with that obtainable by Wooldridge's acetic acid method from the cells. It contains 1·45 per cent. of phosphorus. It does not become viscous on admixture with neutral salts, and the sodium chloride method of preparing nucleo-proteids is not applicable to it. It produces intravascular coagulation.
3. A globulin coagulating at 70° C.
4. An albumin in mere traces, which coagulates at about the same temperature.

Other organic constituents of the liver cells.—Urea, uric acid (especially in birds), xanthine, and hypoxanthine, are found in the liver;[2] leucine and tyrosine are found in cases of acute yellow atrophy, and in phosphorus poisoning.[3] Various other substances have been described as occasional constituents.[4]

A substance called *jecorin*, containing phosphorus ($C_{105}H_{186}N_5SP_3O_{45}$), was separated from the liver by Drechsel.[5] In its properties it somewhat resembles lecithin; it, however, reduces Fehling's solution, which lecithin does not. According to Baldi,[6] it occurs in many other organs—spleen, muscle, brain, etc.

The question of the iron-containing nucleins of the liver (Zaleski's hepatin, Schmiedeberg's ferratin,[7] etc.) is alluded to on p. 69. The iron in the liver is increased in diseases, like pernicious anæmia, which lead to increased destruction of red blood corpuscles; it is normally greater in young (especially new-born) animals than in older ones. Animals appear to enter the world with a store of iron in the liver, and to a less degree in the spleen, which lasts them until they are able to take foods other than milk, which is poor in iron.[8]

[1] *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 806.

[2] Scherer, *Ann. d. Chem.*, Leipzig, Bd. cvii. S. 314; Cloetta, *ibid.*, Bd. xcvii. S. 289.

[3] Sotnitschewsky, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 391; see also Röhmann, *Berl. klin. Wchnschr.*, 1888, S. 43 and 44.

[4] Guanine, inosite, scyllite (Frerichs and Städeler, *Mitth. d. Zürich. natur. Gesellsch.* 1858); cystine (Hoppe-Seyler, "Physiol. Chem.," S. 718); sarcolactic acid, probably formed after death.

[5] *Journ. f. prakt. Chem.*, Leipzig, Bd. xxxiii. S. 435.

[6] *Arch. f. Physiol.*, Leipzig, 1887, Suppl., S. 100.

[7] For recent work in ferratin and iron in the liver, and absorption of iron compounds as food, see F. Vay, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xx. S. 377; Woltering, *ibid.*, Bd. xxi. S. 186; Hall, *Arch. f. Physiol.*, Leipzig, 1896, S. 49, 142; Cloëtta, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, Bd. xxxiii. S. 6; Hochhaus and Quincke, *ibid.*, S. 152; Quincke, *ibid.*, S. 182.

[8] Meyer and Pernou, *Ztschr. f. Biol.*, München, Bd. xxvii. S. 439; Lapicque, *Compt. rend. Soc. de biol.*, Paris, tome xli. p. 435.

Inorganic constituents of the liver.—Oidtmann[1] found 1·1 per cent. of inorganic material in the liver, of which potassium phosphate, as in many other organs, is the most abundant. His numbers per cent. are:—

Potash . . .	25·17	Phosphoric acid .	43·37
Soda . . .	14·17	Sulphuric acid .	0·9
Lime . . .	3·62	Silicic acid . .	0·27
Magnesia . .	0·19	Chlorine . .	2·5
Iron oxide . .	2·75	Manganese, lead, copper traces.	

F. Krüger and Lenz[2] found that the liver cells of the calf contain about 70 per cent. more calcium than in the ox. During the fœtal period there are two maxima and two minima in the amount of calcium, which varies inversely with that of iron. In the liver cells of adult men, Krüger and his assistants[3] found 2·38 of sulphur, 1·28 of phosphorus, and 0·77 of iron per cent. In new-born children the three numbers are respectively 5·56, 1·54, and 0·314.

The spleen.—The percentage of water in the adult human spleen varies from 69·4 to 77·5, the solids, from 31·6 to 22·5, of which 30·1 to 21·6 consist of organic, and from 1·1 to 0·9 of inorganic, matters.[4]

During life the spleen is alkaline. Acidity sets in after death, due to the formation of sarcolactic acid.[5]

The organic constituents of the spleen are proteids and hæmoglobin, xanthine,[6] hypoxanthine, uric acid,[7] glycogen,[8] inosite,[9] scyllite,[10] cerebrin,[11] cholesterin, lecithin, and jecorin.[12] Various fatty acids (formic, acetic, butyric) described by Scherer[13] are, no doubt, derived during the process of distillation from the proteids. Leucine and tyrosine, which are absent from the fresh organ, are often found as a result of putrefactive changes (Hoppe-Seyler). The inorganic constituents are very like those found in the liver, except that sodium are more abundant than potassium salts.[14]

The proteids of the spleen.—Gourlay[15] found that the proteids which can be extracted from fresh spleen resemble those found in lymphoid structures; the most important of these are a cell globulin coagulating at 49°–50° C., and a nucleo-proteid coagulating at 57–60° C. Bottazzi[16] confirms these observations in the main. The nucleo-proteid can be prepared either by Wooldridge's or the sodium chloride method, and, like that obtained from other cellular organs, produces intravascular coagulation.

[1] "Die anorg. Bestandtheile der Leber," Linnich, 1858.
[2] *Ztschr. f. Biol.*, München, 1895, Bd. xxxi. S. 392. [3] *Ibid.*, S. 400.
[4] Oidtmann, *loc. cit.*
[5] Hirschler, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xi. S. 41.
[6] Scherer, *Ann. d. Chem.*, Leipzig, Bd. cvii. S. 314; Städeler, *ibid.*, Bd. cxvi. S. 102; Neubauer, *Ztschr. f. anal. Chem.*, Wiesbaden, Bd. vi. S. 33; Gorup-Besanez, *Ann. d. Chem.*, Leipzig, Bd. xcviii. S. 1; Cloëtta, *ibid.*, Bd. xcix. S. 289.
[7] Scherer, Gorup-Besanez, Cloëtta.
[8] Hoppe-Seyler, "Med. Chem. Untersuch.," Bd. iv. S. 495; Abeles, *Centralbl. f. d. med. Wissensch.*; Berlin, 1876, No. 5.
[9] Cloëtta, Scherer.
[10] Frerichs and Städeler, *Mitth. d. Zurich. natur. Gesellsch.*, 1855.
[11] Hoppe-Seyler. [12] Baldi, *Arch. f. Physiol.*, Leipzig, Suppl., 1887, S. 100.
[13] *Verhandl. d. phys.-med. Gesellsch. zu Würzburg*, Bd. ii. S. 323.
[14] Oidtmann gives the following percentages:—Soda, 35–45; phosphoric acid, 18–30; sulphuric acid, 1·5–2·5; potash, 9–17; oxide of iron, 7–16; silica, 0·2–0·7; lime, 7; chlorine, 0·5–1·3; manganese, copper, lead, traces. For a comparison of the percentage of sulphur and phosphorus in the hepatic and splenic cells at different ages, see F. Krüger, *Ztschr. f. Biol.*, München, 1895, Bd. xxxi. S. 400.
[15] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 23.
[16] *Ann. di chim. e di farm.*, 1895, vol. xxi.

Another point in connection with the spleen relates to the question whether or not proteoses or peptones are obtainable from it; this is important, because Sidney Martin[1] has found that the proteoses of diseases (diphtheria, tetanus, etc.) accumulate in the spleen. v. Jaksch[2] states that normal spleen contains "peptone"; but the careful work of Gourlay, in which he used Devoto's ammonium sulphate method and the alcohol method failed to detect any.

Lymphatic glands.—The capsule yields gelatin and mucin like connective tissue structures generally. The reticular tissue yields reticulin (see p. 72) and gelatin (see p. 70). The chemistry of the cells has been already described (p. 81).

In a lymphatic gland, about two-thirds are water, the remainder solids. The tissue is alkaline during life, and turns acid, due to the development of sarcolactic acid, after death.[3]

Thymus.—This is also principally lymphoid tissue, and the above remarks apply equally well to it. Nothing special is known of the chemistry of the concentric corpuscles. The presence of extractives like xanthine and hypoxanthine has been noted by Scherer, Gorup-Besanez, Frerichs, Städeler, etc., whose writings have been already referred to. Schindler[4] has estimated the "nuclein or alloxuric bases" (see p. 67) obtainable from the thymus of the calf, with the following results:—

Percentage in	Adenine.	Hypoxanthine.	Guanine.	Xanthine.
Fresh tissue . .	0·179	0·0023	0·0075	0·038
Dry tissue . .	1·919	0·218	0·071	0·360

The high percentage of adenine is noteworthy. Like the other organs already described, the reaction, alkaline during life, becomes rapidly acid after death. The acid is sarcolactic acid.[5]

The thyroid.—This organ is also alkaline during life, but becomes acid after death; this is due to sarcolactic acid (Moscatelli).

Various extractives (fatty acids, xanthine, hypoxanthine, etc.) have been found in it by Gorup-Besanez, Scherer, Frerichs, and Städeler. Inosite has been found by Fränkell[6] and by Tambach.[7] The main constituents of the thyroid, however, are proteids, and a proteid-like substance from the colloid material in the acini.

Oidtmann found in the adult thyroid, 82·24 water, 17·66 organic and 0·1 inorganic material per cent. In an infant's thyroid the numbers were 77·21, 22·35, and 0·44 respectively.

The importance of the chemistry of the thyroid arises from the fact that the administration of thyroid extracts has been attended with curative results in cases where the thyroid is absent, or no longer forms the internal secretion which is believed to be necessary for the nutrition of the nervous system.

[1] Goulstonian Lectures, *Brit. Med. Journ.*, London, March 1892.
[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1892, Bd. xvi. S. 243.
[3] Hirschler, *Ibid.*, Bd. xi. S. 41.
[4] *Ibid.*, Bd. xiii. S. 438.
[5] Moscatelli, *ibid.*, Bd. xii. S. 416.
[6] *Wien. med. Bl.*, 1895, No. 48; 1896, Nos. 13, 14, 15.
[7] *Pharm. Centr.-Bl.*, Leipzig, 1896, Bd. iv. S. 119.

Various attempts have been made to discover the active principle in the thyroid which is responsible for its curative properties. Notkin[1] attributes the activity of the gland to its proteid constituents, especially to the one called *thyreoproteid* by Bubnow,[2] which acts after the manner of an enzyme.

Gourlay[3] made a thorough investigation of the proteids obtainable from the organ. His conclusions were as follows:—

1. The only proteid that can be obtained in any quantity from the thyroid is a nucleo-proteid.[4] This may be prepared by either the acetic acid or sodium chloride method, and when intravascularly injected causes thrombosis.

2. This is derived, at any rate partly, from the colloid matter in the acini; it yields no sugar on treatment with dilute mineral acid, and is therefore not a mucin or mucoid. Moreover, the microchemical method of Lilienfeld and Monti shows that it contains phosphorus. The absence of mucin is confirmed by Fränkel and by Hutchison.

3. Small quantities of albumin are also obtainable.

A year later Fränkel[5] separated from the gland a crystalline material, with the formula $C_6H_{11}N_3O_5$, which he called *thyreo-antitoxin*, though the experimental and clinical evidence quoted hardly seem to justify the name.

Roos and Baumann[6] have discovered an iodine-containing material, which occurs chiefly in combination with the proteid of the organ, but partly free. It is remarkable in being insoluble in 10 per cent. hydrochloric acid, a reagent which dissolves all the other substances present. It was previously known that the active substance was very stable. Thyroid feeding is followed by as good results as injection of thyroid extracts; the active substance therefore resists the action of digestive ferments. The substance was named by its discoverers *thyro-iodin*, or *iodo-thyrin*; it contains 9·3 per cent. of iodine, and 0·56 per cent. of phosphorus. It is not probably a derivation of nuclein, but its constitution is not yet known. The amount of iodine per gramme of the organ in human adults varies from 0·3 to 0·9.

Whether this substance is really the important proximate principle in thyroid extracts and by inference in the normal internal secretion of the organ, must still be left to the future. For, though Roos and Baumann state that it acts in every way like thyroid extracts, Gottlieb[7] has been unable to confirm the statement, though possibly, as Auerbach[8] suggests, this is to be attributed to his having used preparations very poor in iodine. Weak points in the theory appear to be the absence of the substance in the thyroids of children, and in some animals like dogs unless they are put on a particular diet (dog biscuits). Small quantities of iodine are found also in the thymus.

[1] *Wien. med. Wchnschr.*, 1895, Nos. 19 and 20; *Virchow's Archiv*, 1896, Bd. cxliv. S. 224. The ferment theory was also urged by White and Davies, *Brit. Med. Journ.*, London, 1892, vol. ii. p. 966.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. viii. S. 1.

[3] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 23.

[4] Morkotun (*Vrach*, St. Petersburg, 1895, No. 37) gives the composition of this nucleo-proteid as—C, 51·46; N, 15·56; P, 0·32; H, 6·94; S, 1·5; O, 24·22 per cent.

[5] *Wien. med. Bl.*, 1895, No. 48; 1896, Nos. 13, 14, and 15.

[6] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxi. S. 19, 319, 481; xxii. S. 1, 18.

[7] *Deutsche med. Wchnschr.*, Leipzig, Bd. xxii. S. 235,

[8] *Centralbl. f. Physiol.*, Leipzig, 1896, Bd. x. S. 133. For various other references to clinical work on this question, see *ibid.*, No. 6. For the influence of iodo-thyrin on metabolism, see F. Voit, *Ztschr. f. Biol*, München, 1897, Bd. xxxv. S. 116.

This discovery of a compound containing iodine in the animal body is a very remarkable one, but is not unique. Almost simultaneously with Baumann's announcement, Drechsel[1] published a research on the horny skeleton of *Gorgonia cavolinii*. Here he found iodine in organic combination, and on decomposition the skeleton yielded a crystalline amido-acid (iodo-gorgonic acid) of uncertain constitution, and with the formula $C_4H_8NIO_2$. Drechsel has also found iodine in the hair of a syphilitic patient, taking iodide of potassium. With reference to the thyroid, he suggests the very reasonable hypothesis that this organ produces more than one active substance, and that the different substances have different actions. He has confirmed the existence both of Baumann's iodo-thyrin and of Fränkel's thyreo-antitoxin, and has further separated out a second crystalline base. Hutchison,[2] however, finds that the proteid-free extracts which contain these bases are physiologically inactive. He finds that the activity is connected with the iodine-containing colloid substance. He distinguishes between the colloid of the acini and the nucleo-proteid of the epithelium lining them. The former is the active constituent, and is by gastric digestion decomposed into two parts. One part is proteid; it contains a little iodine, and has feeble physiological powers. The other part is not proteid, and not nuclein. It is more active, and contains the greater part of the iodine and all the phosphorus of the original colloid.

The suprarenal body.—In this gland, in addition to proteids and the usual extractives and salts (among which potassium phosphate is the most abundant), various other substances have been described, such as hippuric and taurocholic acid,[3] benzoic acid, taurine,[4] and inosite.[5]

The chemistry of the suprarenal is of especial interest because of the work of Schäfer and Oliver[6] on the action of extracts obtained from it. It is now generally believed that the function of the gland is secretory, and that the fatal effects of its removal in animals, or disease in man (Addison's disease), is due to the removal of an internal secretion, and not to auto-intoxication from the non-removal of waste products.[7] The active principle is obtained from extracts of the medulla of the healthy gland; it is absent in advanced cases of Addison's disease.

The earlier observers[8] were inclined to attribute the toxic results of suprarenal injections to neurine. This is not so. Neurine

[1] *Ztschr. f. Biol.*, München, 1896, Bd. xxxiii. S. 83; *Centralbl. f. Physiol.*, Leipzig, Bd. ix. S. 704.

[2] *Brit. Med. Journ.*, London, 1896, vol. i. p. 722; 1897, vol. i. p. 4; *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 474.

[3] Cloez and Vulpian, *Compt. rend. Acad. d. sc.*, Paris, 1857, tome ii. p. 10; *Gaz. méd. de Paris*, 1858, No. 24.

[4] Seligsohn, Diss., Berlin, 1858; Holm, *Journ. f. prakt. Chem.*, Leipzig, Bd. c. S. 150. Stadelmann could not confirm these statements, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. Possibly these substances are absorbed from the neighbouring gall bladder and kidney.

[5] Külz, *Sitzungsb. d. Gesellsch. z. Beförd. d. ges. Naturw. zu Marburg*, 1876, No. 4.

[6] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 230. Some speculations as to the function of the cortex by Auld will be found, *Brit. Med. Journ.*, London, July 4, 1896; Manasse, *Virchow's Archiv*, Bd. cxxxv. S. 263.

[7] The discovery of hæmochromogen in the medulla of the organ by MacMunn (*Proc. Roy. Soc. London*, Bd. xxxix. S. 248) appeared to favour the removal hypothesis.

[8] Pellacani, *Arch. per le sc. med.*, Torino, 1874, vol. iii.; Foà, *ibid.*, 1884, vol. viii.; Marino-Zucco, *Chem. Centr.-Bl.*, Leipzig, 1888; *Untersuch. z. Naturl. d. Mensch. u. d. Thier*, Bd. xiv.; *Arch. ital. de biol.*, Turin, vol. x.

can be obtained from the gland, it is true, but the symptoms of neurine poisoning are different. The active principle has not yet been satisfactorily identified, although its solubilities and many of its reactions have been worked out by Moore,[1] who at first thought it identical with a powerfully reducing substance found only in the medulla of the gland, and first described by Vulpian.[2] The solubilities of this reducing substance are nearly identical with those of the active physiological principle. It gives a dark green or blue colour with ferric chloride, passing through purple to a dark red on the addition of ammonia or sodium carbonate. With chlorine, bromine, or iodine water, peroxide of hydrogen, or alkalis in the presence of oxygen, it gives a rose-red colour, discharged by sulphide of hydrogen or ammonium sulphide. It is insoluble in alcohol, ether, or benzene; it is soluble in water, alcohol *plus* water, and dilute acids. It dialyses freely through vegetable parchment. It is not a proteid, nor a carbohydrate, nor a fat, nor is it affected by gastric digestion.

Manasse,[3] who investigated the composition of the organ without any special reference to the question of its physiological action or the work of Schäfer and his colleagues, states that a reducing substance is present, similar in many of its properties to jecorin (see p. 86). It is, however, not jecorin; the two substances are alike in some of their solubilities, but the material from the suprarenal does not reduce Fehling's solution until after prolonged boiling with sulphuric acid; the sugar formed appears to be dextrose. Moore has, however, been unable to obtain from the suprarenal any substance that reduces Fehling's solution. If one, moreover, compares the percentage composition of Manasse's material with jecorin, the difference is seen to be striking, as in the following table:—

	Jecorin.		Substance from Suprarenals.
	Drechsel.	Baldi.	Manasse.
C . .	51·32–51·64	46·88–46·89	41·43
H . .	8·11– 8·25	7·81– 8·09	7·16
N . .	2·86	4·36– 4·88	0·3
S . .	1·42–1·47	2·14– 2·70	1·8
P . .	2·2 –3·7	2·29– 2·75	4·44
Na .	2·72	5·72	...
O . .	30·10	...	...

S. Fränkel[4] has also made an attempt to identify the active substance, but with no better success than Moore; according to him, the material obtained by Manasse is inactive. Nabarro[5] has investigated the proteids of the organ and found them similar to those of other glandular structures, namely, cell globulin and nucleo-proteid. They appear to be physiologically inactive. In his later work Moore[6] criticises Fränkel's

[1] "Proc. Physiol. Soc.," London, March 1894 (*Journ. Physiol.*, Cambridge and London, vol. xvi. p. i); *ibid.*, March 1895 (*Journ. Physiol.*, Cambridge and London, vol. xvii. p. ix.); *Journ. Physiol.*, Cambridge and London, vol. xvii. p. 230.

[2] *Compt. rend. Acad. d. sc.*, Paris, tomes xliii. and xlv.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 478.

[4] *Wien. med. Bl.*, 1896, Nos. 14, 15, and 16.

[5] "Proc. Physiol. Soc.," London, 1895, *Journ. Physiol.*, Cambridge and London, vol. xvii.

[6] *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 382.

methods and results. He finds that absolute alcohol, which Fränkel used for extracting the active substance from the gland, only dissolves it in traces; and that the prolonged action of alcohol, especially if heat is employed, renders the material physiologically inactive, though it still continues to give the colour reactions enumerated above. He is inclined to consider the substance to be a derivative of piperidine, not of pyrocatechin, as Fränkel supposes. Piperidine certainly produces a marked rise of blood pressure, like suprarenal extract.[1]

Pancreas.—This organ is alkaline during life, and rapidly becomes acid after death. The solids are like those usually obtained from cellular organs, namely, proteids (for the phospho-gluco-proteid separated from the gland by Hammarsten, see p. 64); extractives (guanine,[2] xanthine, hypoxanthine, leucine,[3] tyrosine, uric acid, lactic acid, inosite), and a small proportion of inorganic salts.

Salivary glands.—The submaxillary gland yields proteids, of which the most abundant is a nucleo-proteid;[4] the cells also contain mucinogen, which passes as mucin into the saliva. The parotid cells contain no mucin. A small amount of mucin is, with gelatin, obtainable from the investing connective tissue.

The kidneys.—During life the reaction of renal tissue is alkaline; after death it rapidly becomes acid, especially the medulla.[5]

Gottwalt[6] gives the following table relating to the percentage composition of kidney tissue freed from blood:—

Proteids	11·185 to 12·217 per cent.
Gelatin	0·996 „ 1·849 „
Mucin	Traces.

The following extractives have been obtained by various observers:—xanthine, hypoxanthine, creatine, taurine, leucine, cystin, urea, uric acid, glycogen, and inosite.

The kidney also contains a small proportion of inorganic salts (0·1 to 0·7, Oidtmann).

The proteids of kidney tissue.[7]—These are very like the proteids of other glands, and consist of cell globulin, coagulable by heat at 52° C., and a nucleo-proteid. This is far the more abundant; it coagulates at 63° C.; it may be prepared by either the acetic acid or sodium chloride method. It contains 0·37 per cent. of phosphorus, and produces, like other nucleo-proteids, intravascular coagulation.

The lungs.—The chemical constituents of these organs call for no special notice.[8]

The testis.—Chemically, the testis is mainly composed of proteids,

[1] This was shown independently by Tunnicliffe, *Centralbl. f. Physiol.*, Leipzig, 1897, Bd. x. S. 777.

[2] Scherer, *Ann. d. Chem.*, Leipzig, Bd. cxii. S. 276.

[3] Virchow, Frerichs, and Städeler, see Hoppe-Seyler, "Physiol. Chem.," S. 260. These substances are present in the fresh organ, and are not, as in the spleen, the result of putrefaction.

[4] Hammarsten, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 163.

[5] Halliburton, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 806. Liebermann states that the normal reaction of kidney tissue is acid, *Arch. f. d. ges. Physiol.*, Bonn, Bd. l. S. 55.

[6] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iv. S. 431.

[7] Halliburton, *loc. cit.*

[8] "On Lecithin in Lungs and Sputum," see Zoja, *Gazz. med. di Torino*, 1894, vol. xlv.

or substances allied to proteids; of the latter, nuclein and nucleo-proteids are the most abundant.

As in other cases, the fresh gland is alkaline;[1] the acidity noted by Treskin[2] was probably the result of post-mortem changes. The extractives which have been found are leucine and tyrosine (these are probably post-mortem products); lecithin, cholesterin, and fat (Treskin); creatine;[3] inosite;[4] adenine, xanthine, hypoxanthine, guanine,[5] and other derivatives of nuclein.[6]

The salts present are chiefly chlorides of sodium and potassium (Treskin).

Semen.—The chief chemical constituent of the spermatozoa is nuclein (Miescher, see p. 66). Miescher also prepared a base which he called *protamine*, and to which Piccard[7] ascribed the formula $C_{16}H_{32}N_9O_4$. Another organic substance, akin to a proteid, and containing 4 per cent. of sulphur, was also described by Miescher.

Kossel[8] has examined the protamine from the testis of salmon and sturgeons; he calls it salmine or sturine, according to its origin. He prepared from it various crystalline salts, and a new base, $C_6H_9N_3O_2$, he terms histidine.[9]

Among other substances he prepared from fishes' spermatozoa, was thymin, the substance he had previously got from the nucleic acid of the thymus (see p. 66).

Lecithin, next to nuclein and proteids, is the chief organic substance in spermatozoa.[10] Cholesterin and fat are also fairly abundant. Miescher gives the following percentage for the salmon's spermatozoa:—

Nuclein . .	46·68 per cent.	Lecithin . .	7·47 per cent.
Protamine .	26·76 „	Cholesterin .	2·24 „
Proteids . .	10·32 „	Fat . . .	4·53 „

Miescher continued to work at this subject (salmon's spermatozoa) throughout his life. He, however, never published much beyond his early papers. After his death, Schmiedeberg published an article[11] compiled from his numerous notes. This paper relates to the quantitative composition of the spermatozoa, and gives analyses of the principal substances obtained from them, especially nuclein and protamine. He considers these are in chemical union, thus:

$$\underset{\text{(nucleic acid)}}{7C_{40}H_{45}N_{14}O_{17}(P_2O_5)_2} + \underset{\text{(protamine)}}{C_{16}H_{28}N_9O_2}$$

The heads of the spermatozoa contain 60·73 per cent. of nucleic acid and 19·78 per cent. of protamine. The tails (which are soluble in

[1] Sertoli, *Gazz. med.-vet.*, Milano, 1872, Anno ii.
[2] *Arch. f. d. ges. Physiol.*, Bonn, Bd. v. S. 122.
[3] Schottin, see Hoppe-Seyler, "Physiol. Chem.," S. 773.
[4] Schottin, Külz, *Sitzungsb. d. Gesellsch. z. Beförd. d. ges. Naturw. zu Marburg*, 1876, No. 4.
[5] Schindler, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 438.
[6] Kossel, *ibid.*, 1896, Bd. xxii. S. 172, 188; Hedin, *ibid.*, S. 191.
[7] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. vii. S. 1714.
[8] *Loc. cit.*
[9] Hedin (*loc. cit.*) believes histidine is identical with a base he had previously obtained in his work on the decomposition products of proteids.
[10] Diaconow; see Hoppe-Seyler's "Med. Chem. Untersuch.," Bd. ii. S. 221; iii. S. 405.
[11] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, Bd. xxxvii. S. 100.

saline solutions) contain, proteid, 41·9; lecithin, 31·73; fat and cholesterin, 26·27 per cent. In young spermatozoa some interest attaches to the presence of a proteose which is regarded as the mother substance of protamine. Proteose and protamine both give the biuret reaction.

Charcot's crystals.—These can be obtained from semen on evaporation.[1] They are frequently found in sputum, in the blood, and in other situations, in leucocythæmia. Schreiner[2] considered that they consist of the phosphate of a base he called spermine, C_2H_5N. Ladenburg and Abel[3]

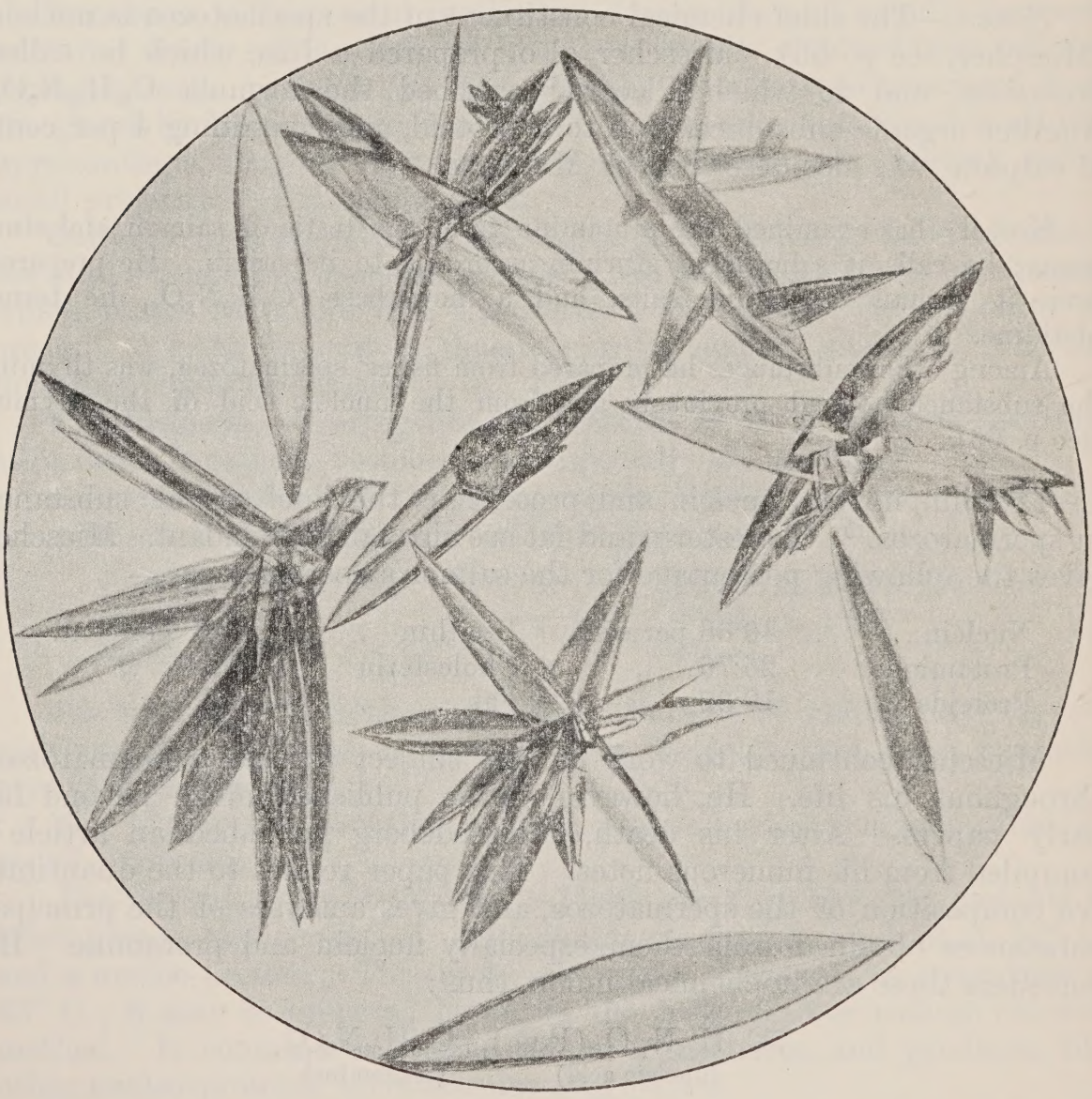


Fig. 12.—Charcot's crystals.

thought they were identical with ethylenimine, which can be prepared artificially from ethylenediamine-hydrochloride. This identity, however, is denied by Majert and A. Schmidt[4] and by Poehl.[5] Poehl gives the formula $C_5H_{14}N_2$ to the base. He states that it is a normal constituent of the testis, ovary, and blood, and that, used as a drug, it has a tonic effect.

Ovary.—The connective tissue element is large, and yields chiefly gelatin and mucin. Proteids and nuclein are derived from the ova and

[1] Böttger, *Virchow's Archiv*, Bd. xxxii. S. 525.
[2] *Ann. d. Chem.*, Leipzig, Bd. cxciv. S. 68.
[3] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxi. S. 758.
[4] *Compt. rend. Acad. d. sc.*, Paris, tome cxv.
[5] *Berl. klin. Wchnschr.*, 1893, No. 36.

other cells present. The corpora lutea are coloured by lutein. This is a lipochrome (see p. 20). Thudichum[1] was the first to point out that it is distinct from hæmatoidin, which is also generally present.

The constituents of eggs are described with the various proteids, etc., of which they are made up.

Muscle.

Skeletal muscle.—A muscle contains, besides the muscular fibres, supporting connective tissue with fat. Each fibre consists of two parts, the sheath or sarcolemma, and the contractile substance which it encloses. The sarcolemma resembles elastin very closely in its solubilities.[2]

The contractile substance is of soft consistency, and contains a large percentage of proteids, and smaller quantities of various extractives and salts. By the use of a press a juice can be squeezed out of perfectly fresh muscles, which is called the *muscle plasma.* Like blood plasma, this coagulates, and the proteid clot is called *myosin*; this occurring within the body after death is the cause of rigor mortis.

Living muscle is alkaline; but after extreme activity, and after death, the reaction becomes acid; this is due in part to the development of sarcolactic acid.

The percentage of water in muscle varies in different animals:[3]—

Man	72 to 74 per cent.	Birds	70 to 76 per cent.
Ox	77 „	Amphibians	76 „ 80 „
Pig	78 „	Fishes	79 „ 82 „
Cat	75 „	Crab	85 „
Fox	74 „	Pecten	79 „ 80 „

In young animals, and during inanition, the percentage of water is greater.

Human muscle has the following average percentage composition:—

Water	73·5 per cent.
Solids	26·5 „
Proteids, including sarcolemma, proteids of connective tissue, vessels, and pigments	18·02 „
Gelatin	1·99 „
Fat	2·27 „
Extractives (creatine, lactic acid, glycogen, etc.)	0·22 „
Inorganic salts	3·12 „

This may be compared with the muscle of a mollusc (*Pecten*):[4]—

Water	79·60 to 80·25 per cent.	
Solids	20·40 „ 19·75 „	
Proteids	15·68 „ 15·04 „	
Glycogen	2·43 „ 1·98 „	
Glycocine	0·71 „ 0·39 „	
Ethereal extractives	0·33 „ 0·24 „	
Inorganic salts	1·26 „ 1·22 „	

[1] *Centralbl. f. d. med. Wissensch.*, Berlin, 1869, Bd. vii. S. 1.

[2] Ewald, *Ztschr. f. Biol.*, München, Bd. xxvi. S. 1.

[3] Schlossberger, "Chem. der Gewebe," Leipzig and Heidelberg, 1856, S. 169; Gorup-Besanez, "Lehrbuch," 1878, S. 676; Hoppe-Seyler, "Physiol. Chem.," S. 636.

[4] Chittenden, *Ann. d. Chem.*, Leipzig, Bd. clxxviii. S. 266.

Muscle considered as meat is the most concentrated and most easily assimilable of the animal nitrogenous foods. It forms our chief source of nitrogen. In 100 parts of nitrogen from beef, 77·4 come from proteid insoluble in water, 10·08 from soluble proteid, and 12·52 from extractives.[1] In addition to the proteids, extractives, and salts contained in muscle, the flesh used as food contains a certain variable percentage of fat, even though all visible adipose tissue is cleaned off. In estimating the amount of fat, Dormeyer[2] recommends that the meat should be subjected to artificial digestion before extraction with ether; flesh then yields an additional 0·75 per cent. of fat.

The following table[3] gives the chief substances in some of the principal meats used as food:—

Constituents.	Ox.	Calf.	Pig.	Horse.	Fowl.	Pike.
Water	76·7	75·6	72·6	74·3	70·8	79·3
Solids	23·3	24·4	27·4	25·7	29·2	20·7
Proteids and gelatin . .	20·0	19·4	19·9	21·6	22·7	18·3
Fat	1·5	2·9	6·2	2·5	4·1	0·7
Carbohydrate	0·6	0·8	0·6	0·6	1·3	0·9
Salts	1·2	1·3	1·1	1·0	1·1	0·8

The flesh of young animals is richer in gelatin than that of old ones; thus 1000 parts of beef yield 6, of veal 50, parts of gelatin (Liebig).

Meat contains four times the amount of proteid present in an equal weight of milk.

The process of cooking meat (after it has been kept to allow *rigor mortis* to pass off) renders the investing connective tissues looser, separates the muscular fibres, and destroys parasitic growths. The muscular fibres themselves, especially if boiled, are rendered more difficult of digestion.

The muscle plasma and the muscle serum.—Kühne[4] was the first to obtain muscle plasma; he used frogs' muscle. The fresh blood-free muscle is frozen and subjected to strong pressure, the expressed fluid (muscle plasma) is filtered; it is found to be syrupy in consistence, and faintly alkaline. As the temperature of the plasma rises to that of the air, it clots, and the myosin, so formed, contracts to a slight extent, squeezing out *muscle serum.* Kühne found this latter fluid to contain—

(1) A proteid coagulating at 45° C.; (2) an alkali albumin;[5] (3) a small quantity of albumin; (4) extractives and salts.

A good many years later, I was successful in repeating these experiments with mammalian muscle,[6] and showed, moreover, that not only

[1] Salkowski, *Centralbl. f. d. med. Wissensch.*, Berlin, 1894.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lxi. S. 341; 1896, Bd. lxv. S. 90; Schulze, *ibid.*, S. 299; 1897, Bd. lxxii. S. 145.

[3] Munk's "Physiologie," Aufl. 4, S. 280.

[4] "Lehrbuch d. physiol. Chem.," S. 272; "Untersuch. ü. das Protoplasma," Leipzig, 1864.

[5] The natural alkali albumins described by older workers are no doubt all globulins.

[6] Halliburton, *Journ. Physiol.*, Cambridge and London, vol. viii. pp. 133–202.

does cold prevent the coagulation of muscle plasma, but, as in the case of blood plasma, admixture with solutions of neutral salts has the same effect. Addition of water to the salted muscle plasma brings about coagulation (an acid reaction making its appearance simultaneously), especially at 40° C., and still more rapidly if solution of "myosin ferment" is added. The myosin ferment was prepared from muscle in the same way as fibrin ferment from blood serum.

Saline extracts of muscle which has undergone rigor mortis, resemble salted muscle plasma very closely; after dilution they undergo coagulation; this may be a re-coagulation of the redissolved myosin, for the acidity of the saline extract is increased by re-coagulation. Some observers, however, regard this phenomenon not as a true re-coagulation, but as a simple precipitation of the myosin by dilution with water.

Myosin may be most readily extracted from muscle by means of ammonium chloride solution, and may be precipitated in a gelatinous form by dialysing away the salt.[1] Elementary analysis[2] gives the following results:—C, 52·79; H, 7·12; N, 16·86; S, 1·26; O, 22·97.

Recent research has shown that calcium salts are essential for the effective coagulation of milk and blood. The facts are not so positive in the case of muscle, but there is some evidence pointing to the existence of an analogy in the three cases.[3]

By fractional heat coagulation, and by their varying solubilities in different salts, I was able to separate four different proteids in the muscle plasma.

(1) A globulin precipitable by heat at 47° C. This is analogous to the cell globulin found in most protoplasmic structures. It is termed *musculin* by Hammarsten. I gave it the name *paramyosinogen.*

(2) A globulin precipitated by heat at 56° C. This is the proteid which is especially acted on by the myosin ferment, and is converted into myosin. I termed it *myosinogen*: both it and paramyosinogen contribute to the muscle clot.

(3) A third globulin precipitated at 63° C. (*myoglobulin*) is contained in the muscle serum.

(4) Small quantities of an albumin (*myoalbumin*), similar in its properties to serum albumin, are also present.

In addition to this, in the case of red muscles, there is hæmoglobin; and if the muscle has been kept warm, and acidity developed, small quantities of proteoses and peptone, which are formed by a process of self-digestion. Brücke showed, many years ago, that muscle contains small quantities of pepsin, doubtless absorbed from the gastric mucous membrane; this becomes active on the onset of acidity. The action of such a ferment within the body will perhaps explain the phenomenon called the *disappearance of rigor mortis*; it is unnecessary to suppose that this is always due to putrefactive organisms,[4] since rigor often disappears before putrefaction sets in. Perfectly fresh muscle contains no proteose or peptone.[5]

Whitfield also investigated the question whether myosin or its precursors are

[1] Kühne and Chittenden, *Ztschr. f. Biol.*, München, Bd. xxv. S. 358.

[2] Chittenden, *ibid.* See also *Stud. Lab. Physiol. Chem.*, New Haven, 1889, vol. iii. p. 116.

[3] Locke, *Journ. Physiol.*, Cambridge and London, vol. xvii. p. 293; other references will be found in this paper.

[4] Cossar Ewart, *Proc. Physiol. Soc.*, London, 1887, p. xxv.

[5] Whitfield, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 487.

of the nature of nucleo-proteids. He found that they are not. He was indeed able to obtain no nucleo-proteid at all from muscle. Pekelharing[1] has taken up the latter question, and by an improved method discovered that muscular tissue does contain a small amount of nucleo-proteid. He points out that on gastric digestion small quantities of nuclein are soluble, if the amount of hydrochloric acid present exceeds 0·1 per cent. Whitfield used water as an extracting agent for any possible nucleo-proteid. Pekelharing points out that the water will soon become acid from sarcolactic acid, and uses dilute (0·15 per cent.) sodium carbonate solution instead. From such an extract the nucleo-proteid can be precipitated by acetic acid. From 543 grms. of flesh he obtained 2 grms. of nucleo-proteid. This substance produces intravascular clotting, and contains 0·7 of phosphorus. The nuclein split off from it contains 3·5 per cent. of phosphorus, and on decomposition yields alloxuric bases, especially xanthine and guanine. Hypoxanthine and adenine were not found. Kossel[2] also failed to get adenine from muscle.

An important research on muscle plasma and its proteids has lately been published by v. Fürth.[3] He obtained the plasma from blood-free muscles by extracting them with physiological saline solution. This extract coagulates spontaneously, and the clotted proteid formed he calls *myogen fibrin* or *myosin fibrin*. The proteids in the muscle plasma he reduces to three, namely, paramyosinogen, 17 to 22 per cent. of the total proteid; myosinogen or myogen, 77 to 83 per cent., and traces of an albumin.[4]

My work is confirmed in its main point, namely, that there are two proteids in the muscle plasma, paramyosinogen and myosinogen, which enter into the formation of the muscle clot; the action of a specific ferment to bring about this change was not specially investigated. The principal new fact made out is, that paramyosinogen passes into the condition of myosin fibrin directly; whilst in the passage of myosinogen into the state of myogen fibrin, there is an intermediate soluble stage coagulable by heat, at the remarkably low temperature of 40° C.[5]

Paramyosinogen is described as a typical globulin, and is regarded as identical with Kühne's myosin which he obtained by dropping muscle plasma into water. Myosinogen is described as differing from a globulin in some particulars, and is spoken of as a proteid *sui generis*. Myoglobulin is not regarded as a separate proteid, but as part of the myosinogen which has escaped coagulation. The phenomenon regarded by Chittenden and myself as re-coagulation of myosin is considered to be a simple re-precipitation of globulin. Whitfield's work on the absence of peptones and proteoses is confirmed.

The muscle plasma from fishes' and crabs' muscle contains another proteid, called *myo-proteid*. It gives the usual proteid reactions, and is readily digested by gastric juice; though precipitated by a removal of the salts by dialysis, it is not coagulable by heat. It is precipitable by acetic acid, but is neither a mucin nor a nucleo-proteid.

In his second paper, v. Furth treats of (1) the properties (solubilities,

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 245.

[2] *Ibid.*, 1886, Bd. x. S. 248.

[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1895, Bd. xxxvi. S. 231; also *ibid.*, 1896, Bd. xxxvii. S. 389.

[4] J. H. Milroy (*Arch. f. Hyg.*, München u. Leipzig, 1896, Bd. xxv. S. 154) has also made quantitative estimations of the various muscle proteids coagulable at different temperatures.

[5] If the reader refers to my memoir on "Muscle Plasma," he will find, on p. 186, that I accidentally noted this fact, though I failed to appreciate its meaning. In frogs' muscle plasma there is a considerable amount of this soluble myogen fibrin in a "preformed" condition (v. Fürth). The separation of the muscle proteids by fractional heat coagulation fits in exactly with Brodie and Richardson's work on heat rigor; as the temperature of a muscle is raised, successive shortenings occur at the coagulation temperature of each proteid (*Proc. Roy. Soc. London*, 1897, vol. lxi. p. 77).

effect of numerous reagents, etc.) of myosinogen and paramyosinogen; (2) the influence of blood serum in hindering the coagulation of the muscle plasma; and (3) the action of various chemical substances on living muscle.

Involuntary muscle.—Our chemical knowledge of involuntary muscle is of a fragmentary nature. Like voluntary muscle, the heart becomes rapidly rigid after death, and simultaneously acid,[1] from the formation of sarcolactic acid. Both paramyosinogen and myosinogen are present in the muscle cells of the heart, and myosin is the result of coagulation. In the stomach and uterus, rigor has been observed, but in other forms of plain muscle it is difficult to recognise. A proteid coagulating at 56° C., has been obtained from all kinds of unstriped muscle. In a muscular tumour of the uterus, Kossel[2] found the one coagulating at 45° C. (paramyosinogen) to be absent.

The reaction of unstriped muscle is normally alkaline.[3] Lehmann[4] found small quantities of lactic acid in the muscular substance of the stomach after death. There is, however, no marked change in the reaction after death, as in striated muscle. Du Bois-Reymond[5] observed in the stomach and intestines of birds that after death the muscular walls were still alkaline.

Myohæmatin.—Though hæmoglobin is the pigment of the red muscles, MacMunn[6] considers that the specific pigment of ordinary muscle is myohæmatin, one of the most widely distributed of the colouring matters which he has described under the name *histohæmatins.* The histohæmatins have only been observed by the spectroscope; they have not been separated out by chemical processes. They often occur in animals that possess no hæmoglobin. As they undergo changes in their absorption bands, by oxygenation and reduction, it is believed that they are respiratory in function. The spectrum of these substances is somewhat like that of hæmochromogen; and Levy, working under Hoppe-Seyler,[7] has gone so far as to say that myohæmatin is hæmochromogen produced by the methods used to render the muscle transparent. The resemblance is not absolute, but is specially close in what MacMunn calls modified myohæmatin. This is produced by artificial gastric digestion; or it can be obtained in the following way:—The muscle is chopped finely and covered with ether for some days. A yellow lipochrome derived from the fat between the muscular fibres[8] passes into solution, and below this floats a red juice, which on filtration gives the spectrum in question.

Until myohæmatin and the other histohæmatins are examined by methods other than spectroscopic, it is impossible to pronounce positively on the point of dispute between MacMunn and Levy. The fact that in the last experiment described, the muscles, even if they are full of blood, yield no longer any hæmoglobin, points to hæmoglobin as the source of the myohæmatin; whether this substance can be produced in the muscles *intra vitam* must be left to the future to decide.[9]

The extractives of muscle.—These are—(*a*) *Nitrogenous*, namely,

[1] Boruttau, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 513.
[2] Quoted by Hoppe-Seyler, "Physiol. Chem.," S. 669.
[3] Bernstein (Kühne's "Lehrbuch," S. 332) found the actively contracting muscles of Anodon acid.
[4] "Lehrbuch," Bd. iii. S. 73.
[5] *Monatsb. d. k. preuss. Akad. d. Wissensch. zu Berlin*, 1859, S. 312.
[6] *Phil. Trans.*, London, 1886; *Journ. Physiol.*, Cambridge and London, vol. vii.
[7] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. MacMunn's reply is in the same vol., S. 497.
[8] Halliburton, *Journ. Physiol.*, Cambridge and London, vol. vii. p. 325.
[9] K. Mörner (*Nord. med. Ark.*, Stockholm, Festband, 1897) states that muscle pigment is hæmoglobin which spectroscopically shows slight differences from that obtained from blood.

creatine, creatinine, xanthine, hypoxanthine, carnine, carnic acid, uric acid, urea, taurine, and inosinic acid. (*b*) *Non-nitrogenous*, namely, fats, glycogen, inosite, dextrose, and lactic acids.

Creatine and creatinine. — Creatine can be crystallised out by evaporating aqueous extracts of meat from which proteids and salts have been previously removed; on heating it with mineral acids it is converted into creatinine. The relationship of these two substances is shown by the following equation:—

$$\underset{\text{(creatine)}}{C_4H_9N_3O_2} - H_2O = \underset{\text{(creatinine)}}{C_4H_7N_3O}$$

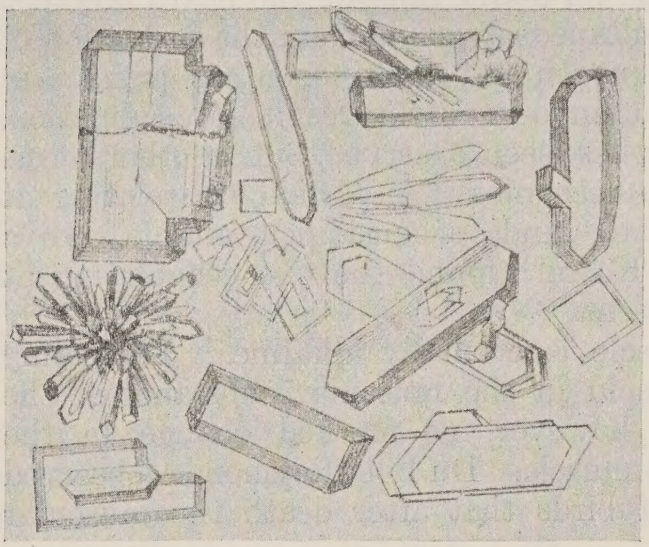


FIG. 13.—Creatine crystals.—After Kühne.

According to Voit,[1] the quantity of creatine in the voluntary muscles varies from 0·2 to 0·3 per cent. This increases during starvation.[2] Involuntary (cardiac and plain) contains less than voluntary muscle.[3]

The compound with zinc chloride which creatinine forms (Fig. 15) has been generally used for isolating it from urine, and other places where it occurs. My own experience with this method has shown that for quantitative purposes it is most uncertain; and this no doubt accounts for the different results obtained by different observers. Thus Neubauer denies the existence of creatinine in muscle altogether; Voit, Sarokin,[4] and Monari[5] say that it increases during muscular activity, while Nawrocki[6] states that it does not. Much more certain results are obtained by the use of G. S. Johnson's method, in which he precipitates the creatinine as a compound of mercury.[7] This method, which has received the powerful recommendation of Hoppe-Seyler,[8] may be used to identify creatinine when it is present in very small quantities, as in the blood.[9] The microscopic appearance of the precipitate is shown in Fig. 16. By

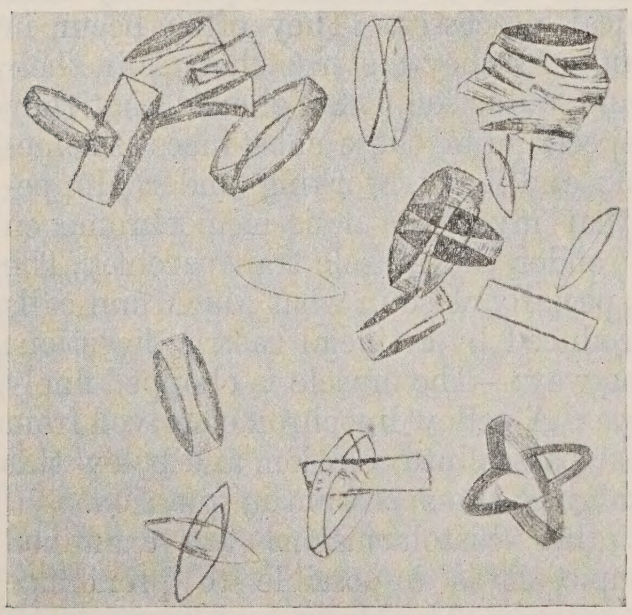


FIG. 14.—Creatinine crystals.—After Kühne.

[1] *Ztschr. f. Biol.*, München, Bd. iv. S. 77.
[2] Demant, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 387.
[3] Voit, *loc. cit.*; Lehmann, "Lehrbuch," Bd. iii. S. 73.
[4] *Virchow's Archiv*, Bd. xxvii. [5] *Gazz. chim. ital.*, vol. xvii. p. 367.
[6] *Centralbl. f. d. med. Wissensch.*, Berlin, 1865, S. 417.
[7] *Proc. Roy. Soc. London*, vol. xlii. p. 365; xlii. p. 493; l. p. 28. Johnson here points out that there are several isomeric varieties of creatinine, differing in reducing power, etc. In his process he is careful to employ no heat; otherwise the creatinine is transformed into a non-reducing variety, or even may be changed into creatine.
[8] "Handbuch. d. physiol. chem. Analyse," 1893, 7th edition, S. 142.
[9] Colls, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 107.

this means Johnson showed that creatinine (a different creatinine from urinary creatinine) is more abundant in muscle than creatine, which is usually almost entirely absent. This unexpected result has been confirmed by Kemmerich.[1] Creatinine is readily changed into creatine by the action of putrefactive micro-organisms.

Xanthocreatinine ($C_5H_{10}N_4O$), *crusocreatinine* ($C_5H_8N_4O$), *amphicreatine* ($C_9H_{19}N_7O_4$), and *pseudoxanthine* ($C_4H_5N_5O$) are leucomaines stated by Gautier[2] to be present in small quantities.

Xanthine, hypoxanthine, and uric acid are found in small quantities only; the numbers given are as follows:—xanthine, 0·0026 per cent.;[3] hypoxanthine, 0·022–0·026;[4] uric acid, traces.[5] Uric acid is more abundant in the muscles of reptiles (alligators). The crystalline forms of some of the compounds of xanthine and hypoxanthine are given in Fig. 17.

Carnine is a crystalline base ($C_7H_8N_4O_3+H_2O$), originally found by Weidel[6] in large quantities (1 per cent.) in American meat extracts, but since found in the flesh of many animals.[7] It is probably closely related to the members of the uric acid group just mentioned.

FIG. 15.—Creatine-zinc chloride crystals. —After Kühne.

Urea.—It is generally stated that muscle contains little or no urea. This statement is chiefly due to the fact that it was until recently a matter of difficulty to separate urea, when only present in small quantities, from other nitrogenous bases. In some animals, however, the muscular tissue contains a fairly large amount of urea. This is the case with the muscles of arthropods.[8] Städeler and Frerichs[9] were the first to discover that the organs, including the muscles, of Selachian fishes are rich in urea. This was confirmed in the case of Selachian embryos by Krukenberg,[10] and more recently in the adult animals by Schröder.[11] In two varieties of dog-fish, the mean percentage of urea in the blood was 2·61, in muscle 1·95, and in liver 1·36. Schrœder explains this by the fact that the kidneys are sluggish in these animals. By a new method, Schöndorff[12] has been able to satisfactorily establish the existence of a small quantity of urea in the muscles of mammals; Kaufmann[13] gives the percentage

[1] Kemmerich, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xviii. S. 409.
[2] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xxii. S. 335.
[3] Scherer, *Ann. d. Chem.*, Leipzig, Bd. cvii. S. 314.
[4] Neubauer, *Ztschr. f. anal. Chem.*, Wiesbaden, Bd. vi. S. 33.
[5] Meissner, *Ztschr. f. rat. Med.*, Leipzig, Bd. xxxi. p. 144.
[6] *Ann. d. Chem.*, Leipzig, Bd. clviii. S. 353.
[7] Krukenberg and Wagner, *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1883, No. 4. See also *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xi. S. 340.
[8] Krukenberg, *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1881, Bd. iv. S. 33; "Vergleich. physiol. Vorträge," 1886, S. 313.
[9] *Journ. f. prakt. Chem.*, Leipzig, 1858, Bd. lxxiii. S. 48; *ibid.*, Bd. lxxvi. S. 58.
[10] "Vergleich. physiol. Vorträge," 1886, S. 314.
[11] *Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xiv. S. 576; Krukenberg, *Centralbl. f. d. med. Wissensch.*, Berlin, 1887, No. 25.
[12] *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lxii. S. 332. For the method employed, see *ibid.*, S. 1.
[13] *Arch. de physiol. norm. et path.*, Paris, Sér. 5, tome vi.

as 0·027 to 0·07. On the other hand, it must be stated that such an experienced chemist as Nencki[1] is still unable to discover any urea in muscle.

Taurine is found in the muscles of horses, fishes, and molluscs. In fishes Limpricht[2] found 1·06 per cent.

Glycocine is found to the extent of 0·39 to 0·71 per cent. in the non-striated muscles of molluscs.[3]

Protic acid is an acid of doubtful nature, described by Limpricht in fishes' muscles.

Inosinic acid ($C_{10}H_{14}N_4O_{11}$) was first described by Liebig, and

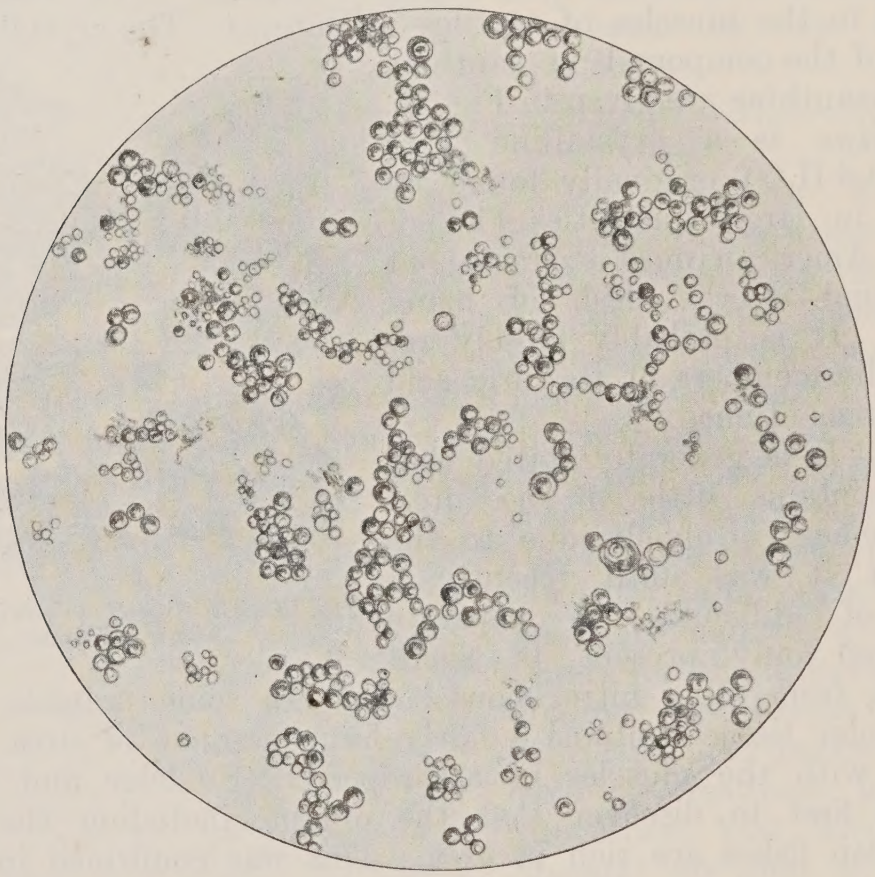


FIG. 16.—Spherical compound of mercury and creatine.—After G. S. Johnson.

estimated (0·005 to 0·02 per cent.) by Creite.[4] According to Fränkel,[5] it is closely related to carnic acid, to be immediately described.

Lecithin and its decomposition products are present in small quantities, and are probably derived from the nerves supplying the muscle.[6] Small quantities of cholesterin are found as well.

Carnic acid (*Fleischsäure*) is the name given by Siegfried[7] to a constituent of muscle, the discovery of which is of great importance. He first prepared it from muscle extracts by means of ferric chloride; the compound so obtained is called *carniferrin*; this contains phosphorus as

[1] Nencki and Kowaski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1895, Bd. xxxvi. S. 395.

[2] *Ann. d. Chem.*, Leipzig, Bd. cxxvii. S. 185; cxxxiii. S. 300.

[3] Chittenden, *ibid.*, Bd. clxxviii. S. 266.

[4] *Ztschr. f. rat. Med.*, Leipzig, Bd. xxxvi. S. 195.

[5] "Zur Kenntniss der Zerfallproducte des Eiweisses," Wien, 1896.

[6] Hoppe-Seyler, "Physiol. Chem.," S. 647.

[7] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1894, Bd. xxvii. S. 2762; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxi. S. 360.

well as iron. By means of baryta water, carnic acid ($C_{10}H_{15}N_3O_5$) was separated out from it. In muscle, this acid is combined with phosphorus as phospho-carnic acid. *Carnic acid itself is identical with antipeptone.* This discovery itself shows that our views concerning the hemi- and anti-products of digestive proteolysis will need revision. Carnic acid is a comparatively simple substance, of low molecular weight, and of acid reaction. It is free from sulphur, and gives most of the proteid

FIG. 17.—Compounds of xanthine and hypoxanthine, by means of which these substances may be isolated and identified.—After Kühne.

a. Hypoxanthine silver nitrate, $C_5H_4N_4O.AgNO_3$.
b. Hypoxanthine nitrate, $C_5H_4N_4O.HNO_3$.
c. Hypoxanthine hydrochloride, $C_5H_4N_4O.HCl$.
d. Xanthine silver nitrate, $C_5H_4N_4O_2.AgNO_3$.
e. Xanthine nitrate, $C_5H_4N_4O_2.HNO_3$.
f. Xanthine hydrochloride, $C_5H_4N_4O_2HCl$.

tests; it does not give Millon's reaction. This discovery will no doubt form an important clue in the problem of proteid constitution. This announcement of Siegfried's has been fully confirmed by Balke,[1] who has prepared many compounds and derivatives (oxycarnic acid, $C_{30}H_{41}N_9O_{15}$; oxylic acid, $C_{18}H_{28}N_4O_8$; and various crystalline metallic salts of these acids), and has devised a method for its estimation.[2] It

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 248.
[2] Balke and Ide, *ibid.*, Bd. xxi. S. 380.

has been further confirmed by Fränkel,[1] who finds that pure amphopeptone is also sulphur-free.

Phosphocarnic acid has a complicated molecule; it yields on decomposition carnic acid, carbonic anhydride, succinic acid, sarcolactic acid, and a strongly reducing carbohydrate. Siegfried compares it to nuclein; but nucleins yield proteid on decomposition; phosphocarnic acid yields carnic acid (antipeptone) instead; he suggests the term *nucleon* for it. The percentage of this substance in human muscle is 0·1–0·2. In new-born children the muscles contain less (0 to 0·06 per cent.[2]

A phosphocarnic acid is also found in milk, but differs from that in muscle by yielding fermentation lactic acid instead of sarcolactic acid on decomposition.[3]

Krüger[4] has found that on hydrolysis and simultaneous oxidation by means of ferric chloride, phosphocarnic acid gives off carbonic anhydride; no other substance in muscle extracts does this. He therefore looks upon it as the material in muscle which during muscular activity gives off carbonic anhydride without using up oxygen. This is a conclusion that requires serious consideration and renewed research before it can be accepted, but it is another indication of the importance of Siegfried's work.

We now pass to the non-nitrogenous extractives:—

Glycogen.—This substance may be extracted from muscle by hot water[5]; or by dilute potash[6]; the latter reagent effects a much more thorough extraction. Cramer,[7] using Külz's method, found that different groups of muscles contain varying amounts of glycogen, but that corresponding muscles of the two sides of the body contain the same amount. In the heart, glycogen is unequally distributed in the different regions (Cramer). The average percentage of glycogen in fresh heart muscle is, however, about the same as in voluntary muscle, though it disappears after death (being converted into sugar as in the liver) more rapidly than in skeletal muscle.[8] Glycogen also occurs in other involuntary muscles.[9]

The glycogen in muscle during life varies in quantity. The following are the principal causes of variation:—

1. Starvation.—The muscle glycogen disappears during inanition, but much more slowly than the hepatic glycogen.[10] Luchsinger[11] stated that the glycogen of the heart muscle disappears still less quickly, but Aldehoff (using Külz's method) could not confirm this.

[1] *Loc. cit.*

[2] M. Müller, *Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. xxii. S. 561.

[3] K. Wittmaack (*ibid.*, S. 567) gives the percentage of nucleon in human milk as 0·124; in cows' milk, 0·056, and in goats' milk, 0·11. Blumenthal (*Virchow's Archiv*, Bd. cxlvi. S. 65) gives the percentage in cows' milk as 0·05.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 95.

[5] Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1871, Bd. lxiii. Abth. 2, S. 214; Nasse, *Arch. f. d. ges. Physiol.*, Bonn, Bd. ii. S. 97.

[6] Abeles, *Med. Jahrb.*, Wien, 1877, S. 551; Külz, *Ztschr. f. Biol.*, München, Bd. xxii. S. 161. See also Schmelz, *ibid.*, Bd. xxv. S. 180.

[7] *Ibid.*, Bd. xxiv. S. 67.

[8] Boruttau, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 513.

[9] In the stomach, Brücke, *loc. cit.*; in the plain muscles of gastropods, Chittenden, *Ann. d. Chem.*, Leipzig, Bd. clxxviii. S. 266; Bizio, *Atti. r. Ist. Veneto di sc., lett. et arti*, 1866, Sér. 3, tome i.

[10] Weiss, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. lxiv.; Aldehoff, *Ztschr. f. Biol.*, München, Bd. xxv. S. 137.

[11] Dissertation, Zurich, 1875.

2. Work.—During work the glycogen disappears, being perhaps transformed into sugar and the products of its combustion, of which lactic acid may be an intermediate one[1] (Weiss, Manché, Monari). This loss of glycogen is shown by numerous analyses, of which the following from Manché will serve as a type:—

	Percentage of Glycogen in Limb at rest.	Percentage of Glycogen in opposite Limb, which was made to contract for 23-65 minutes.	Percentage loss of Glycogen in Tetanised Limb.
1	0·1277	0·114	12·76
2	0·2287	0·1942	15·09
3	0·2267	0·1917	15·44

3. Removal of liver.—Minkowski,[2] Laves,[3] and Schmelz[4] find that after removal of the liver the muscle glycogen rapidly diminishes. Some observers,[5] however, consider that the muscles have a glycogenic function apart from that of the liver.

4. Cutting the nerve of a muscle causes an accumulation of glycogen in the muscle so paralysed.[6]

5. Cutting the tendon of a muscle produces the same effect.[7]

6. Ligature of the artery of a muscle leads to a decrease in its glycogen, especially if œdema follows the operation, the accumulated lymph leading to saccharification (Chandelon, Manché).

Sugar.—During life the sugar in muscle is at a minimum; it increases after death as the glycogen disappears. The sugar is not maltose, as Nasse[8] supposed, but dextrose, as Meissner[9] suggested; the work of Panormoff[10] with the phenylhydrazine reaction has placed this beyond doubt. Small quantities of *dextrin* are found as an intermediate product.[11]

Inosite.—The occurrence of this substance in voluntary muscle has been noted by Scherer[12] and Limpricht; in unstriated muscle by Lehmann; and in heart muscle, where it is more abundant than in skeletal muscle, by Boruttau.[13]

Fat.—This is always obtained from muscle, though whether any occurs in the true muscular substance apart from the entangled adipose tissue, it is difficult to say. Dormeyer[14] finds that after muscle has been subjected to preliminary gastric digestion, ether extracts 8·5

[1] Weiss, *loc. cit.*; Manché, *Ztschr. f. Biol.*, München, Bd. xxv. S. 163; Monari, *Chem. Centr.-Bl.*, Leipzig, 1889, Bd. ii. S. 372.
[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxiii. S. 139.
[3] Inaug. Diss., Königsberg, 1886.
[4] *Ztschr. f. Biol.*, München, Bd. xxv. S. 180.
[5] Prausnitz, *Ibid.*, Bd. xxvi. S. 377; Schmelz, *loc. cit.*
[6] Chandelon, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xiii. S. 626; Manché, *loc. cit.*
[7] E. Krauss, *Virchow's Archiv*, Bd. cxiii. S. 315.
[8] "Zur. Anat. u. Physiol. der quergestreiften Muskel," Leipzig, 1882.
[9] *Nachr. v. d. k. Gesellsch. d. Wissensch. u. d. Georg-Aug.-Univ.*, Göttingen, 1861, S. 206; 1862, S. 157.
[10] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvii.
[11] Nasse, *loc. cit.*; Limpricht, *loc. cit.*
[12] *Ann. d. Chem.*, Leipzig, Bd. lxxvii. S. 322.
[13] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 513.
[14] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxv. S. 90.

per cent. more of the total fat obtainable; without such preliminary digestion, extraction with ether is useless for quantitative purposes. E. Bogdanow[1] believes that the fat which is thus soluble in ether with difficulty is a real constituent of the muscle plasma, and states that it is richer in volatile fatty acids than that from the surrounding connective tissues. For "Adipocere," see p. 20.

Lactic acids.—Among the oxypropionic acids with the empirical formula $C_3H_6O_3$, one called hydracrylic acid, or ethylene lactic acid, $CH_2(OH).CH_2.COOH$, is not found in the body. Small quantities of this material were formerly described[2] as occurring in muscle extracts, but this is not the case; the acid mistaken for it was acetyl-lactic acid $H_3CH(C_2H_3O_2)COOH$.[3]

The remaining lactic acids are stereochemical isomerides of ethylidene lactic acid. They are three in number, and differ in optical activity, and in the solubility, optical activity, and amount of crystallisation water in their zinc, calcium, and lithium salts.[4]

Their formula is $CH_3.CH(OH).COOH$. The differences between them are due, according to the theory of Bel and Van't Hoff, and as the expression stereochemical implies, to the space relationships of the atoms.

The three isomerides are—

(*a*) The optically inactive acid. This is the ordinary fermentation lactic acid, which occurs in milk when it turns sour; it has been found in small quantities in muscle,[5] in the grey matter of the brain,[6] and in some cases of diabetic urine. Its most characteristic salts are—

Zinc lactate $Zn(C_3H_5O_3)_2 + 3H_2O$; soluble in fifty-three parts of water at 15°; in six parts at 100° C.; almost insoluble in alcohol.

Calcium lactate, $Ca(C_3H_5O_3)_2 + 5H_2O$; soluble in 9·5 of cold, and in all proportions in boiling water. Insoluble in cold alcohol.

(*b*) Dextrorotatory lactic acid.—This is paralactic, or sarcolactic acid. This is the lactic acid *par excellence* of muscle.[7] It is found in the blood,[8] particularly after muscular activity.[9] It is found in the urine after muscular activity,[10] during diminution of oxidation processes,[11] in phosphorus poisoning, and after extirpation of the liver.[12] It is found as noted when we considered them, in many organs and tissues after death. Its best known salts are—

Zinc sarcolactate, $Zn(C_3H_5O_3)_2 + 2H_2O$. Soluble in 17·5 parts of water at 15° C., and in 96·4 parts of boiling 98 per cent. alcohol.

[1] *Arch. f. d. ges. Physiol.*, Bonn, Bd. lxv. S. 81.

[2] Wislicenus, *Ann. d. Chem.* Leipzig, 1873, Bd. clxvii. S. 302.

[3] Siegfried, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1889, S. 2711.

[4] On lithium lactates, see Hoppe-Seyler and Araki, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 365.

[5] Heintz, *Ann. d. Chem.*, Leipzig, 1871, Bd. clvii. S. 314.

[6] Gschleidlen, *Arch. f. d. ges. Physiol.*, Bonn, 1873–4, Bd. viii. S. 71.

[7] Liebig, *Ann. d. Chem.*, Leipzig, 1847, Bd. lxii. S. 326; Wislicenus, *ibid.*, S. 302.

[8] Gaglio, *Arch. f. Physiol.*, Leipzig, 1886, S. 400; Irisawa, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvii. S. 340.

[9] Spiro, *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 111; v. Frey, *Arch. f. Physiol.*, Leipzig, 1885, S. 557.

[10] Colasanti and Moscatelli, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1887, S. 212; Marcus, *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxix. S. 425.

[11] Araki, *Ztschr. f. physiol. Chem.*, Strassburg, Bde. xv., xvi., xvii., and xix.

[12] Minkowski, *Centralbl. f. d. med. Wissensch.*, Berlin, 1885, No. 2; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xxi. S. 40; Marcuse, *loc. cit.*; Nebelthau, *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 123.

Calcium sarcolactate, $Ca(C_3H_5O_3)_2 + 4$ or $4\frac{1}{2}$ H_2O. Soluble in 12·4 parts of cold water and in all proportions of boiling water or alcohol.

These salts are levorotatory, though the free acid is dextrorotatory.

(*c*) Levorotatory lactic acid. This is produced by the fermentation of cane-sugar by means of a special kind of bacillus,[1] and is also found in cultures of Gaffky's typhoid bacillus in a solution of sugar and peptone.[2] Very little is known about it yet.

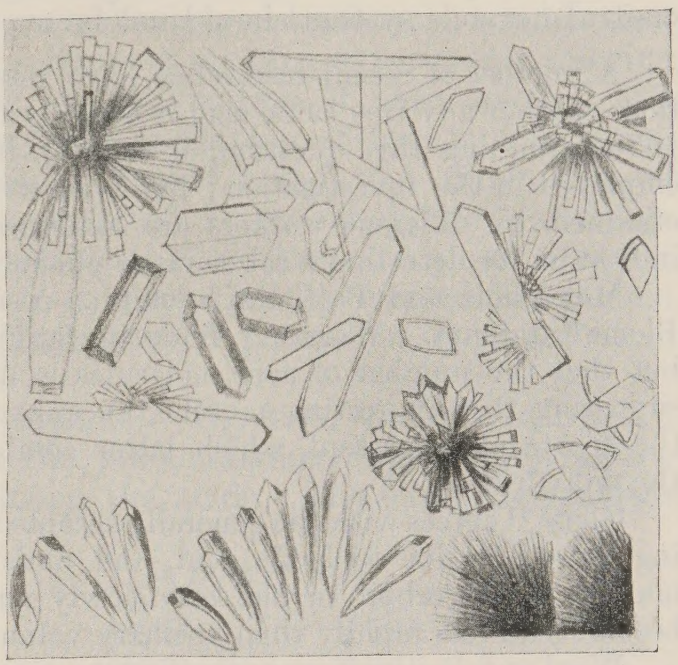


FIG. 18.—Zinc sarcolactate.—After Kühne.

In all cases where three isomerides exist, as in the present case—one optically inactive, one levorotatory, and the third dextrorotatory—it should be understood that strictly speaking there are only two isomerides, one dextro- the other levorotatory, the third or inactive variety being a compound of the other two. This was first shown by Pasteur[3] in connection with racemic acid, which is optically inactive. By appropriate methods of crystallisation it can be separated into two varieties of tartaric acid, one dextrorotatory, the other levorotatory.

Another method of separating an optically inactive material into its optically active components, has been alluded to on p. 32, in connection with glutaminic acid and leucine. It consists in allowing moulds, like *Penicillium glaucum*, to grow in a solution of the inactive compound; one only of its active components is destroyed by the mould, and the other remains untouched. In the case of optically inactive lactic acid, the question has been attacked by the method of crystallisation of various of its compounds, particularly of those with strychnine, and also of zinc ammonium lactate.[4]

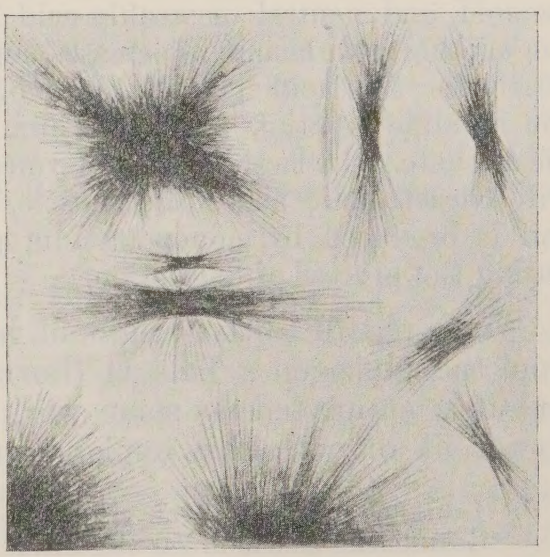


FIG. 19.—Calcium sarcolactate.—After Kühne.

The mode of formation of lactic acid in muscles has been the subject of numerous researches. That the acid is sarcolactic acid has been

[1] Schardinger, *Monatsh. f. Chem.*, Wien, Bd. xi.

[2] Blachstein, *Arch. de sc. biol.*, St. Petersbourg, tome i. p. 199.

[3] *Ann. de. chim.*, Paris, Sér. 2, tome xxiv. p. 442; xxviii. p. 56; *Compt. rend. Acad. d. sc.*, Paris, tome xxxvi. p. 26; xxxvii. p. 162; *Ann. d. Phys. u. Chem.*, Leipzig, Bd. lxxx. S. 127; xc. S. 498, 504.

[4] Purdie and Walker, *Trans. Chem. Soc. London*, 1892, p. 754; 1893, p. 1143.

stated by Berzelius,[1] Du Bois-Reymond,[2] Kühne,[3] and Heidenhain.[4] It may be readily detected in an ethereal extract by Uffelmann's reaction.[5]

Lactic acid is formed, not only after death, but also on activity during life; it is doubtless one of the acid products the accumulation of which produces *fatigue*,[6] though the possibilities of basic products being also produced and causing fatigue by their influence on the central nervous system should not be overlooked.[7]

A number of recent researches have, however, thrown doubt on the question whether any free lactic acid is actually formed under these circumstances. In determining this question, it is very important to know the indicator employed in the investigation; but even with the same indicator the results obtained by different workers are sometimes discordant. One of the best indicators for detecting weak acids is phenolphtalein.

Moleschott and Battistini[8] found a rise of acidity during rigor, while Blome[9] did not. Warren[10] finds in fatigue that the acidity is increased, but that the number of acid molecules is diminished. This is explained by supposing that in resting muscle the anhydride, and in contracting muscle the free acid, is present, which latter combines with twice as much base as the anhydride.

Gleiss[11] agrees with the generally accepted view, that the acidity of contracting muscle is due to lactic acid, and finds that the slowly contracting red muscles of the rabbit, or the very slowly contracting muscles of the tortoise, become acid less rapidly than ordinary voluntary muscles.

Weyl and Seitler[12] were the first to point out that the increase of acidity may be at least in part due to acid potassium phosphate, produced from the alkaline phosphate by the development of new phosphoric acid from organic compounds, like lecithin and nuclein. Irisawa[13] takes a similar view in reference to the acidity of dead organs like the liver and pancreas. The most careful work in this direction, however, is that of Röhmann.[14] He used lacmoid and turmeric as indicators, and found that fresh muscle is alkaline to lacmoid, and neutral or weakly acid to turmeric. During tetanus and rigor, the alkalinity to lacmoid decreases, and the acidity to turmeric increases. He attributes the acid reaction to monopotassium phosphate (KH_2PO_4), and the alkaline reaction to dipotassium phosphate (K_2HPO_4), and to sodium bicarbonate. If lactic acid is formed, none is free. He admits that ether will extract lactic acid from muscle, but it will do so from alkaline muscle, and is produced by monopotassium phosphate turning it out of combination during the process of extraction.

With regard to the origin of lactic acid, O. Nasse believes it comes from the glycogen. This is the simplest view of the matter to take, and it is supported by some work of Ekunina.[15] Many facts, however, do not fit in with this explanation; and the view very generally held

[1] "Lehrbuch d. Chem.," vol. vi. p. 557.
[2] "Gesammelte Abhandl. zur allgemein. Muskel und Nerven Physik," Leipzig, 1877.
[3] "Untersuch. ü. das Protoplasma," Leipzig, 1864.
[4] "Mechanische Leistung," Leipzig, 1864, S. 143.
[5] A dilute solution of ferric chloride and carbolic acid, which is violet, is turned yellow by a trace (1 in 10,000) of lactic acid (*Ztschr. f. klin. Med.*, Berlin, Bd. viii. S. 392).
[6] Ranke, "Tetanus," Leipzig, 1865, p. 350.
[7] A. Mosso, *Trans. Internat. Med. Cong.*, Berlin, 1890.
[8] *Arch. ital. de. biol.*, Turin, vol. viii. p. 90.
[9] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1890, Bd. xxviii. S. 113. Blome's results have been much criticised by Röhmann; *Arch. f. d. ges. Physiol.*, Bonn, 1892, Bd. l. S. 84, *ibid.*, 1893, Bd. lv. S. 589.
[10] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxiv. S. 391.
[11] *Ibid.*, Bd. xli. S. 69.
[12] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vi. S. 557.
[13] *Ibid.*, Bd. xvii. S. 340.
[14] *Loc. cit.*
[15] *Journ. f. prakt. Chem.*, Leipzig, N.F., Bd. xx.

is that the acid arises from the decomposition of complex molecules, of which proteid forms a part. It is quite possible that the lactic acid may originate in both ways.

The idea that the acid has a proteid origin was mooted by Kühne[1] in some of his earliest observations. He showed that not only is the acid formed during rigor mortis, but also during the heat-coagulation of myosin. Böhm[2] supported the proteid origin of lactic acid, and his view was endorsed by Hoppe-Seyler.[3] Some of my own experiments showing the development of acid during the coagulation of pure myosin,[4] and Latham's theoretical views[5] on the constitution of the proteid molecule, tend in the same direction. Araki[6] found that diminution of oxidation in the body, such as is produced by the inhalation of carbonic oxide, leads to the appearance of lactic acid (and sometimes albumin and sugar) in the urine. This is accompanied by increase in proteid katabolism; and this again, as Hammarsten[7] points out, is in favour of the same view.

Inorganic constituents of muscle.—The total ash is from 1 to 1·5 per cent. In it may be noted the predominance of potash among the bases, and of phosphoric acid among the acids. The following analyses are by Bunge:[8]—

	In parts per 1000.	
	I.	II.
K_2O . . .	4·654	4·160
Na_2O . . .	0·770	0·811
CaO . . .	0·086	0·072
MgO . . .	0·412	0·381
Fe_2O_3 . . .	0·057	...
P_2O_5 . . .	4·644	4·58
Cl . . .	0·672	0·70
SO_3 . . .	...	0·10

More recent work on this question is by J. Katz.[9] The flesh of a large number of animals was investigated. The following figures give the minimum and maximum in 1000 parts of fresh flesh —:K, 2·4 to 4·6; Na, 0·3 to 1·5; Fe, 0·04 to 0·25; Ca, 0·02 to 0·39; Mg, 0·18 to 0·37; P (from phosphates), 1·22 to 2·04; (from lecithin), 0·13 to 0·48; (from nuclein), 0·09 to 0·32; Cl, 0·32 to 0·8.

Chemical changes accompanying the contraction of muscle—The physiology of muscular contraction, the influence of muscular work in metabolism, the gases of muscle, and other problems, will be studied in other portions of this work. It may not be inappropriate here, however, to conclude this section by stating briefly the main facts, having a chemical bearing, relating to changes accompanying muscular contraction. The changes are in kind similar to those which occur in

[1] *Arch. f. Anat. u. Physiol.*, Leipzig, 1859, S. 795; "Myologische Untersuch.," Leipzig, 1860, p. 184.

[2] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxiii. S. 44. In a later paper (*ibid.*, 1890, Bd. xlvi. S. 265) Böhm reaffirms his position in reference to some criticisms of Werther (*ibid.*, S. 53).

[3] "Physiol. Chem.," S. 666, 667.

[4] *Journ. Physiol.*, Cambridge and London, 1887, vol. viii. p. 154. These results, however, are criticised by v. Fürth.

[5] *Brit. Med. Journ.*, London, 1886, vol. i. p. 630.

[6] *Loc. cit.* (Note 11, p. 106).

[7] "Physiol. Chem," 3rd German edition, S. 332.

[8] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ix. S. 60.

[9] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 1–85.

muscles during so-called rest; there is an exaggeration of the normal "chemical tone" of the tissue, and an explosive liberation of energy.

1. *Change in reaction.*—The muscle becomes acid; this is generally believed to be due to the production of sarcolactic acid. The views of Röhmann and others in relation to this question (see p. 108) deserve, however, careful consideration.

2. *Changes in the proteid.*—There is no marked and immediate increase of urea in muscular activity, though recent work tends to show that proteid katabolism is increased, and that the increase in urea leaves the body the next day or the day after. The main work, however, appears to fall on the non-nitrogenous part of the muscle, as evidenced by the immediate and large increase in the amount of carbonic anhydride that leaves the muscle. Hermann's theory of muscular contraction assumes that the change is similar in kind to that which occurs on death, though less in degree. On death, he assumes that the hypothetical molecule he terms *inogen*[1] is split into carbonic anhydride, sarcolactic acid, and myosin. But anything like the formation of a clot of myosin has never been observed in living contracting muscle.

3. *Changes in the extractives.*—During tetanus the extractives soluble in water decrease, and those soluble in alcohol increase.[2] This appears to be chiefly explicable by the disappearance of glycogen, and appearance of sugar and lactic acid.

4. *Changes in the gases.*—Hermann's theory just referred to was largely the outcome of his failure to discover oxygen among the gases of muscle. The oxygen used in the formation of carbonic anhydride must therefore be held in complex union within the muscle. On contraction, as on the occurrence of rigor mortis, the amount of carbonic anhydride given off is increased. The amount of oxygen absorbed from the blood is also raised, but not in proportion; hence the fraction $\frac{\text{carbonic anhydride exhaled}}{\text{oxygen absorbed}}$ rises. (See more fully "Respiration").

5. *Production of reducing substances.*—Resting muscle oxidises pyrogallic acid; tetanised muscle does not. A solution of nitrites passed through contracting muscle is changed into one of nitrates, and the colour of solutions of indigo sulphate is altered in the same way as by reducing agents.[3] A. Schmidt[4] arrived at the same conclusion from the examination of the venous blood of tetanised muscle, but what the reducing substances are that are produced is quite unknown.

Electrical organs.—From the torpedo organ, Weyl[5] extracted, probably from the mucous fluid between the plates, a "torpedo mucin." This, however, yields no reducing sugar. A small quantity of gelatin and a globulin (coagulated by heat at 55°–60°) were also obtained.[6] The tissue, like muscle, becomes acid and less transparent after

[1] The nearest approach to Hermann's theoretical substance, inogen, is Siegfried's phosphocarnic acid (see p. 103).

[2] Helmholtz, *Arch. f. Anat. u. Physiol.*, Leipzig, 1845, S. 72; Ranke, "Tetanus," Leipzig, 1865; Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, Bd. iii. S. 574.

[3] Grützner, *ibid.*, Bd. vii. S. 255; Gscheidlen, *ibid.*, Bd. viii. S. 506.

[4] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. xx.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vi. S. 525.

[6] Krükenberg was unable to obtain myosin ("Weitere Untersuch. zur vergleich. Muskelchem." Vergleich. physiol. Studien, 2 Reihe, Abth. 1, S. 143-7).

death.[1] Weyl[2] found the percentage of water in the muscles of torpedo to be 77·5; in the electrical organ, 89. He was also able to separate a number of organic substances from the organ, similar to those occurring in muscle and nerve, such as creatine, xanthine, lecithin, fat, cholesterin, fatty acids, and inosite. Frerichs and Städeler found urea. In another research, Weyl[3] found that excitation of the organ produced an increased formation of phosphoric acid in it.

The Skeletal Tissues.

Most of the chemical substances occurring in the connective tissues (collagen, elastin, mucin, fat) have been already described (see pp. 69–72). There are still a few to be discussed, which will be most conveniently done under the heads—Bone, Tooth, Cartilage, and Notochord.

Bone.—Bone differs from most other tissues in its high percentage of mineral matter. It contains 46·7 per cent. of water,[4] of which Aeby[5] considers 11 or 12 are in a state of loose chemical combination, analogous to water of crystallisation.

The composition of undried bone without separation of marrow or blood is given by Hoppe-Seyler thus: —

Water, 50·00 per cent.	Ossein, 11·40 per cent.
Fat, 15·75 „	Bone earth, 21·85 „

Zalesky's analyses of dried macerated bone are as follows: —

	Human Bone.	Bone of Ox.	Bone of Guinea-Pig.
Organic constituents . .	34·56	32·02	34·70
Inorganic „ . .	65·44	67·98	65·30

Fossil bones analysed by Fremy[6] show a smaller percentage of organic matter.

The organic constituents of bone are ossein or collagen, small quantities of elastin from the lining of the lacunæ and canaliculi,[7] proteids, and nuclein from the cells, and a small quantity of fat even after the removal of all the marrow. The absence of mucin in compact bone is noteworthy, showing that the ground substance is entirely replaced by calcareous matter.[8] Marrow, however, yields mucin.[9] The inorganic constituents of bone are calcium phosphate, calcium carbonate, calcium chloride, calcium fluoride, magnesium phosphate, and small quantities of sulphates and other chlorides.

[1] Boll, *Arch. f. Anat. u. Physiol.*, Leipzig, 1893, S. 99; Du Bois-Reymond found that the electrical organ of *Malapterurus* also becomes acid on activity.
[2] *Monatsb. d. k. Akad. d. Wissensch.*, Berlin, April 1881.
[3] *Arch. f. Anat. u. Physiol.*, Leipzig, 1884, *Physiol. Abth.*, S. 316.
[4] Lukjanow, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 339.
[5] *Centralbl. f. d. med. Wissensch.*, Berlin, 1871, No. 14.
[6] *Ann. de chim.*, Paris, Sér. 3, tome xliii. p. 47.
[7] This substance is not keratin, as Brosicke supposed. See H. E. Smith, *Ztschr. f. Biol.*, München, Bd. xix. S. 469.
[8] R. A. Young, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 803.
[9] Rustiksky, *Centralbl. f. d. med. Wissensch.*, Berlin, 1872, S. 562.

From a large number of analyses, Hoppe-Seyler gives the following figures representing percentages of the total ash:—

Ca.	PO_4.	CO_3.	Fl.	Mg.	Cl.
38·49	54·46	6·24	1·28	0·44	0·19

From his own numbers, Zalesky has calculated the probable composition of the mineral constituents of bone as follows:—

Calcium phosphate . . .	83·889
„ carbonate . . .	13·032
Calcium in combination with fluorine, chlorine, etc.	0·350
Fluorine	0·229
Chlorine	0·183

Hoppe-Seyler considered that the characteristic inorganic ingredient of bone, dentine, and enamel is one analogous to *apatite*. Apatite has the formula $Ca_{10}Fl_2(PO_4)_6$, or $Ca_{10}Cl_2(PO_4)_6$. Very small quantities of these substances, however, occur in bone; the chief compound is one in which CO_3 takes the place of the Fl_2 or Cl_2, namely, $Ca_{10}CO_3(PO_4)_6$. See, however, Gabriel's researches below.

Tooth.—The calcareous tissues of tooth are dentine, enamel, and crusta petrosa. The last named is bone; dentine is chemically similar to bone. Enamel, though epithelial in origin, may be conveniently taken here.

Dentine.—This consists of water 10 per cent., and solids 90 per cent. The solids are organic and inorganic. The organic solids are less abundant than in bone. They consist of collagen and elastin; the latter form the lining of the dentinal tubules. From Aeby's analyses, Hoppe-Seyler gives the following table:—

$Ca_{10}CO_3(PO_4)_6$ . . .	72·06 per cent.
$MgH(PO_4)$	0·75 „
Organic substances . .	27·70 „

Enamel.—This is the hardest tissue in the body. Hoppe-Seyler's quantitative analyses give the following mean result:—

$Ca_{10}CO_3(PO_4)_6$ . . .	96·00 per cent.
$MgHPO_4$	1·05 „
Organic substances . .	3·60 „

Various other investigators give numbers varying from 2 to 10 per cent. of organic matter. This they estimate by loss on ignition. Tomes,[1] however, has recently shown that this loss is chiefly if not wholly due to water. On attempting to estimate the organic matter directly, none was found, or a quantity too small to be weighed.

Gabriel[2] has recently worked at the question of the constitution of the mineral matter of bones and teeth. Some of his conclusions do not accord with the older work of Hoppe-Seyler. He finds that the constituents are water, lime, magnesia, potash, soda, phosphoric acid, carbonic anhydride, chlorine, and fluorine. The quantities of lime and phosphoric acid, which are the most abundant constituents, vary

[1] *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. 217; *Trans. Odont. Soc. Gr. Brit.*, London, 1896. p. 114.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 257.

but little, and are proportional to each other; the amounts of magnesia and carbonic anhydride are also proportional the one to the other. The amount of potash is greater than that of soda. The amount of chlorine is very small, and is greater in the teeth (0·21 per cent.) than in bone. Fluorine is a minimal constituent of both[1]; as a rule, not more than 0·05 per cent. is present.

Water is present in two forms; one part passing off at 300°–350° C. is similar to water of crystallisation; the other part is only expelled by fusion with silicic acid, and is an expression of the basicity of the phosphate, and is called water of constitution or acidic water.

The composition of the ash finds its simplest expression in the formula, $Ca_3(PO_4)_2+Ca_5HP_3O_{13}+Aq$, in which 2 to 3 per cent. of the lime is replaced by magnesia, potash, and soda, and 4 to 6 per cent. of the phosphoric acid by carbonic anhydride, chlorine, and fluorine. The limit of variation is, however, small, and the differences between bone ash and tooth ash are not greater than those between the ash of different bones.

The notochord.—Sternberg[2] found that neither gelatin nor chondrin is obtainable from the notochord, and Neumann[3] that the cells stain with iodine as though they contained glycogen. Kossel[4] obtained a considerable supply of material from large lampreys, and found that it contains 95–96 per cent. of water; this contrasts strongly with cartilage, and corresponds with what one finds in other embryonic tissues. The amount of ash is 0·85 per cent. The amount of glycogen constitutes from 12 to 15 per cent. of the solids present; the high percentage of this substance is another feature common to embryonic structures. There is not much more than a trace of proteid matter soluble in water. Gelatin, collagen, and mucin are all absent; the bulk of the solid matter is an insoluble proteid easily digested by artificial gastric juice; it yields no sugar on treatment with mineral acids.

Cartilage.—The following analyses by Hoppe-Seyler exhibit the relative proportions of the chemical constituents of human hyaline cartilage. In 1000 parts—

	Costal Cartilage.	Articular Cartilage.
Water	676·6	735·9
Solids	323·3	264·1
Organic solids	301·3	248·7
Inorganic solids	22·0	15·4
Potassium sulphate (in a hundred parts of ash)		26·66
Sodium sulphate	,, ,,	44·81
Sodium chloride	,, ,,	6·11
Sodium phosphate	,, ,,	8·42
Calcium phosphate	,, ,,	7·88
Magnesium phosphate	,, ,,	4·55

The organic solids consist in small part of those in the cells, which are of the usual proteid nature, together with small quantities of fat and glycogen, demonstrable by micro-chemical means; but the

[1] For recent estimations of fluorine in bone and teeth by Carnot's method (*Compt. rend. Acad. d. sc.*, Paris, tome cxiv. p. 750), see Gabriel, *Ztschr. f. anal. Chem.*, Wiesbaden, Bd. xxxi. S. 522; and Waampelmeyer, *ibid.*, Bd. xxxii. S. 550.

[2] *Arch. f. Physiol.*, Leipzig, 1881, S. 105.

[3] *Arch. f. mikr. Anat.*, Bonn, Bd. xiv. S. 54.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xv. S. 331.

great bulk of the organic solids is derived from the matrix of the cartilage.

In fibrocartilage, the hyaline matrix is pervaded either by white fibres (white fibrocartilage), or by yellow fibres (yellow or elastic fibrocartilage).

In contrast with true bone, the analysis (by Frémy) of the calcified cartilage of the ray may be here given:—

Ash per cent. . .	. 30·00	Calcium carbonate .	. 4·3
Calcium phosphate .	. 27·7	Magnesium phosphate, traces.	

The matrix of hyaline cartilage.—The organic basis of the matrix was formerly described as *chondrigen*; and just as gelatin is obtained from collagen on boiling, so *chondrin* is obtained by boiling chondrigen. Chondrin, like gelatin, gelatinises on cooling a solution of it made with warm water, but in many of its reactions it differs from gelatin.

Elementary analyses of chondrin, however, showed very great discrepancies, and Morochowetz[1] arrived at the conclusion that chondrin is not a chemical unit but a mixture of gelatin and mucin. This conclusion has been more recently amplified by C. T. Mörner,[2] who worked under the superintendence of Hammarsten.

The matrix contains four substances—(1) collagen, (2) an albuminoid, (3) chondromucoid, and (4) chondroitin-sulphuric acid. Of these constituents the last two, with perhaps a little collagen, lie around the cells, forming what Mörner calls chondrin balls; they correspond to the mucin of Morochowetz, or hyalogen of Krukenberg, and are coloured blue by methyl-violet. They lie in the meshes of a network composed of collagen and mucoid, which is stainable by tropæolin.

These four constituents may be separated as follows. The mucoid and chondroitin-sulphuric acid are dissolved out with 0·2 to 0·5 per cent. solution of potash; the collagen is dissolved out by hot water, being converted into gelatin in the process; the albuminoid remains undissolved.

(1) *The collagen* differs from ordinary collagen in only containing 16·4 per cent. of nitrogen.

(2) *The albuminoid*, which is found only in late adult life, is a proteid-like substance of an insoluble nature. It contains loosely combined sulphur. It differs from elastin in its high percentage of sulphur (see p. 73).

(3) *Chondromucoid.*—This substance has the following percentage composition:—C, 47·3; H, 6·42; N, 12·58; S, 2·42; O, 31·28 (Mörner). The sulphur is loosely combined. Chondromucoid gives the ordinary proteid reactions. On decomposition, it yields the usual decomposition products of proteids, with chondroitin-sulphuric acid in addition; this latter substance is, on further decomposition, broken up into sulphuric acid and a reducing substance. Schmiedeberg[3] regards chondromucoid as a union of proteid with chondroitin-sulphuric acid.

(4) *Chondroitin-sulphuric acid.*—This substance was called chondroitic acid by Bödeker[4] and Krukenberg[5] (who classed it among his hyalins,

[1] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, Part 5, Bd. i.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xii. S. 396; *Skandin. Arch. f. Physiol.*, Leipzig, Bd. i. S. 210.

[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 355.

[4] *Ann. d. Chem.*, Leipzig, 1861, Bd. cxvii. S. 111.

[5] *Ztschr. f. Biol.*, München, Bd. xx. S. 307.

see p. 64). It was first prepared in a pure condition by Mörner, and its constitution made out by that observer and by Schmiedeberg. It is partly found as such in the cartilaginous matrix, but most originates from the decomposition of chondromucoid.

Mörner found that the sulphur in it was all in the form of ethereal hydrogen sulphate; hence the name chondroitin-sulphuric acid. It is almost, but not quite, characteristic of cartilage. Mörner[1] separated it from twenty different varieties of cartilage, from cartilaginous tumours, and also from the tunica intima of the aorta,[2] but from no other tissue or organ of the body.[3]

Schmiedeberg ascribes to it the formula $C_{18}H_{27}NSO_{17}$. On decomposition, the first products are sulphuric acid, and a nitrogenous substance *chondroitin*.

$$\underset{\text{(chondroitin-sulphuric acid)}}{C_{18}H_{27}NSO_{17}} + \underset{\text{(water)}}{H_2O} = \underset{\text{(sulphuric acid)}}{H_2SO_4} + \underset{\text{(chondroitin)}}{C_{18}H_{27}NO_{14}}$$

Chondroitin is a gummy material, and a monobasic acid. On hydration it yields acetic acid, and a new nitrogenous body called *chondrosin*.

$$\underset{\text{(chondroitin)}}{C_{18}H_{27}NO_{14}} + 3\underset{\text{(water)}}{H_2O} = \underset{\text{(acetic acid)}}{C_2H_4O_2} + \underset{\text{(chondrosin)}}{C_{12}H_{21}NO_{11}}$$

Chondrosin is also gummy, and a monobasic acid. It reduces Fehling's solution even more strongly than dextrose; it is dextrorotatory, and is the reducing substance which so many previous chemists have obtained in an impure form from cartilage. On further decomposition it yields glycuronic acid (see p. 5) and glucosamine (see pp. 9 and 75).

Nervous Tissues.

General composition.—The amount of water varies. It is present in larger amount in the grey than in the white matter, in early than in adult life, in the brain than in the spinal cord, in the spinal cord than in nerves. These facts are illustrated by the following table[4]:—

Portion of Nervous System.	Percentages of Water.						
	In Fœtus (W.).	Age, 20–30 (W.).	Age, 70–90 (W.).	(B.)	(P.)	(M.)	(R.)
Grey matter of brain	87–92	83	84	85	81	81	86
White ,,		69	72	70	68		70
Spinal cord . .	...	...	...	73–76		68	...
Nerves . . .	...	...	...	64–72		57	...

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 357.

[2] *Upsala Läkaref. Förh.*, Bd. xxix.

[3] Oddi (*Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxxiii.) states he has obtained it from livers which had undergone amyloid degeneration.

[4] In the above table, (W.) refers to Weisbach (Hofmann's "Lehrbuch d. Zoochemie," Wien 1876, S. 121); (B.) to Bernhart (Gamgee's "Physiol. Chem.," vol. i. p. 446); (P.) to Petrowsky (*Arch. f. d. ges. Physiol.*, Bonn, Bd. vii. S. 367); (M.) to Moleschott (Charles, "Physiol. Chem.," p. 335); and (R.) to de Regibus (*Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xiv. S. 346).

Solids.—The solids may be divided into the following classes:—

(*a*) Proteids.—These comprise a very considerable percentage of the solids, especially in the grey matter (over 50 per cent.).

(*b*) Neurokeratin and nuclein.

(*c*) Phosphorised constituents; especially protagon and lecithin.

(*d*) Cerebrins.—Nitrogenous substances of unknown constitution.

(*e*) Cholesterin.—Especially abundant in white matter.

(*f*) Extractives.—Creatine,[1] xanthine,[2] hypoxanthine,[2] inosite,[3] lactic acid,[3] leucine,[3] uric acid,[3] and urea.

(*g*) Gelatin and Fat.—From the adherent connective tissue.

(*h*) Inorganic salts.—The total mineral matter varies according to different writers from 0·1 to 1 per cent.

Geoghegan[4] gives the following figures in parts per thousand of brain:—

Total ash . .	2·9	to 7·1	Chlorine . . .	0·4	to 1·2
Potassium . .	0·6	„ 1·7	PO_4 . . .	0·9	„ 2·0
Sodium . .	0·4	„ 1·1	CO_3 . . .	0·2	„ 0·7
Magnesium . .	0·0	„ 0·07	SO_4 . . .	0·1	„ 0·2
Calcium . .	0·005	„ 0·02	$Fe(PO_4)_2$ . .	0·01	„ 0·09

The grey matter is stated by Schlossberger to be richer in total ash than the white, but poorer in phosphates; Petrowsky, on the other hand, found more phosphates in grey than in white matter.

The following table gives some typical quantitative analyses which have been made of the proportion in which the principal solids occur in different nervous structures:—

Portion of Nervous System.	Proteids.	Lecithin.	Cholesterin and Fat.	Cerebrins.	Neuro-keratin.	Other Organic Matters.	Salts.
Grey matter of ox brain[5]	55·37	17·24	18·68	0·53	6·71		1·45
White matter of ox brain[5]	24·72	9·90	51·91	9·55	3·34		0·57
Spinal cord[6] .	23·8	75·1					1·1
Human sciatic nerve[7]	36·8	32·57	12·22	11·30	3·07	4·0	...

The quantitative work I[8] have done on this question may be sum-

[1] Müller, *Ann. d. Chem.*, Leipzig, Bd. ciii. S. 141; Städeler, *Journ. f. prakt. Chem.*, Leipzig, Bd. lxxii. S. 256.

[2] Städeler, *Ann. d. Chem.*, Leipzig, Bd. cxvi. S. 102; Scherer, *ibid.*, Bd. cvii. S. 314.

[3] Müller, *loc. cit.*; see also Strecker, *Ann. d. Chem.*, Leipzig, Bd. cv. S. 316.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. i. S. 330.

[5] Petrowsky, *loc. cit.*

[6] Moleschott, *loc. cit.*

[7] Josephine Chevalier, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. x. S. 97.

[8] Halliburton, *Journ. Physiol.*, Cambridge and London, 1893, vol. xv. p. 90.

marised in the following table of mean analyses. The organs were from adult human beings, dogs, cats, and monkeys:—

	Water.	Solids.	Percentage of Proteids in Solids.
Grey matter of cerebrum . .	83·467	16·533	51
White ,, ,, . .	69·912	30·088	33
Cerebellum	79·809	20·191	42
Spinal cord as a whole . .	71·641	28·359	31
Cervical cord	72·529	27·471	31
Dorsal cord	69·755	30·245	28
Lumbar cord	72·639	27·631	33
Sciatic nerves	61·316	38·684	29

This table illustrates the fact that the amount of grey matter, of water, and the percentage of proteid in the solids, vary directly the one with the other. This is very well seen in the different regions of the spinal cord. The percentage of proteid in the white matter of the brain is a little higher than in the spinal cord; this exception is perhaps to be explained by the high percentage of neurokeratin[1] in white matter, which, according to the methods used, would be included with the proteids.

Reaction of nervous tissues.—Heidenhain[2] and Gscheidlen[3] state that the normal reaction of the axis cylinder is alkaline; on death or on long-continued activity the reaction becomes acid. They further state that the grey matter is acid even during life. O. Langendorff[4] found the reaction of the central nervous system alkaline during life; the alkalinity rapidly diminishes after death, or on stoppage of the circulation. S. Moleschott and Battistini[5] found both central and peripheral portions of the nervous system acid, especially the grey matter; this was increased by activity.

In my own work I found in animals that the fresh tissues were invariably alkaline, but they became rapidly acid, especially the grey matter. In the human brains I received from the post-mortem room the reaction of the grey matter was always, of the white matter often, acid. This I put down to changes after death, for at least twenty-four hours had always elapsed since death.

The acidity is due to lactic acid; but, according to Müller and Gscheidlen, it is not sarcolactic acid but the fermentation lactic acid (optically inactive ethylidene-lactic acid). Müller also obtained traces of formic acid.

Proteids of nervous tissues.—The large quantity of these, especi-

[1] The percentage of neurokeratin is in grey matter, 0·3; in white matter, 2·2 to 2·9; and in nerve, 0·3 to 0·6 (Kühne and Chittenden, *Ztschr. f. Biol.*, München, Bd. xxvi. S. 291).

[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, S. 833.

[3] *Arch. f. d. ges. Physiol.*, Bonn, Bd. viii. S. 171.

[4] *Neurol. Centralbl.*, Leipzig, 1885, No. 14; *Centralbl. f. d. med. Wissensch.*, Berlin, 1886, No. 25.

[5] *Arch. ital. de biol.*, Turin, vol. viii. p. 90; *Chem. Centr.-Bl.*, Leipzig, 1887, S. 1224.

ally in the grey matter, has been already alluded to. Petrowsky, in the investigation just mentioned, describes a globulin somewhat resembling myosin, and an albumin especially abundant in grey matter which is coagulated at a temperature of 75° C. Baumstark,[1] in a more recent research, speaks of the chief proteid matter in nervous tissue as resembling casein; this is so, for it is a nucleo-proteid. My own conclusions[2] on the subject are as follows:—

The proteids present are three in number. The first is a globulin, coagulated by heat at 47° C., and analogous to the cell globulin derivable from nearly all cellular tissues. The second and most abundant is nucleo-proteid. In a saline extract of nervous tissues it is mixed with the other proteids; attempts to prepare it by the sodium-chloride method failed. It may, however, be prepared in large quantities by precipitating an aqueous extract of brain by weak acetic acid (Wooldridge's method). The supply obtainable from white matter is small. It is coagulated at 56°–60° C.; it contains 0·5 per cent. of phosphorus, and gives the general reactions of nucleo-proteids, production of intravascular coagulation included. The third proteid is a globulin, coagulated by heat at 70°–75° C., and analogous to a similar globulin separable from liver cells (see p. 86). Peptone, proteose, myosin, and albumin are not obtainable.

Protagon.—In the year 1865, Liebreich[3] separated from the brain a material he called protagon; he further found that, when decomposed by baryta water, it yielded two acids—stearic acid and glycero-phosphoric acid—and a base choline.

Hoppe-Seyler, and Diaconow[4] working under Hoppe-Seyler's direction, denied the existence of this substance, and considered that it was a mere mechanical mixture of lecithin with a nitrogenous non-phosphorised substance called cerebrin. Diaconow's analyses were, however, far from convincing.

The subject was taken up in this country by Gamgee and Blankenhorn,[5] who showed that protagon is a perfectly definite crystalline substance of constant elementary composition. They also showed that even prolonged treatment with alcohol and ether will not extract lecithin from protagon, as alleged by Diaconow. When protagon is digested with alkalis it yields cerebrin or cerebrins, and the decomposition products of lecithin. This work has been confirmed by Baumstark,[6] Ruppel,[7] and Kossel and Freytag.[8]

Protagon is prepared as follows:—The brain is digested with alcohol at 45° C.; the extract is filtered warm, and cooled to 0° C. It then deposits a white precipitate of protagon mixed with cholesterin, which is dissolved out by means of ether. The protagon is dried, redissolved in warm alcohol, and crystallises out on cooling. The empirical formula, calculated from their analytical results, is given as $C_{160}H_{308}N_5PO_{35}$ by Gamgee and Blankenhorn.

[1] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ix. S. 145.
[2] *Journ. Physiol.*, Cambridge and London, 1893, vol. xv. p. 100.
[3] *Ann. d. Chem.*, Leipzig, Bd. cxxxiv. S. 29.
[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, S. 97.
[5] *Journ. Physiol.*, Cambridge and London, vol. ii. p. 113; Gamgee's "Physiol. Chem.," vol. i. p. 427.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ix. S. 329.
[7] *Ztschr. f. Biol.*, München, Bd. xxxi. S. 86.
[8] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvii. S. 431.

The percentage composition is seen in the following table:—

Elements.	Liebreich.	Gamgee and Blankenhorn.	Baumstark.	Kossel.	Ruppel.		Calculated from Formula.
					Ox.	Human.	
C .	66·74	66·39	66·48	66·25	66·29	66·51	66·45
H .	11·74	10·69	11·12	11·13	10·75	10·88	10·66
N .	2·80	2·39	2·35	3·25	2·32	2·55	2·42
P .	1·23	1·068	1·02	0·97	1·13	1·138	1·07
S .	...	...	...	0·51	0·096	...	...
O .	...	19·462	18·701	...	...	...	19·40

An elaborate research by Thudichum[1] led him to the conclusion that there are three groups of phosphorised substances in the brain, which he termed kephalines (very soluble in ether), myelines (less soluble in ether), and lecithins (characterised by their extreme instability). In each of these ill-defined groups several members with their empirical formulæ are described. Thudichum's work has been so far confirmed by that of Kossel, in that he has shown that protagon is not a single substance, but that there is more than one protagon. They yield either one or two or perhaps three derivatives (cerebrosides), called cerebrin, kerasin or homocerebrin, and encephalin; and, further, probably several lecithins are obtainable from the different protagons. The constitution of lecithin is discussed on p. 22, and there it will be seen that the existence of several lecithins (*i.e.* containing different fatty acid radicles) is mooted. The protagons, according to Kossel, resemble each other in the following points:—

1. They contain carbon, hydrogen, nitrogen, oxygen, and phosphorus. Elementary analysis gives practically the same results as those obtained by other observers. But the existence of sulphur in some varieties of protagon is a new point.
2. By oxidation with nitric acid they yield higher fatty acids (palmitic and stearic).
3. By the action of boiling sulphuric or hydrochloric acid a reducing carbohydrate is formed.
4. By the action of alkalis they yield cerebrosides (formerly called cerebrins).
5. The cerebrosides are the source of the reducing carbohydrate mentioned above.
6. The carbohydrate formed is galactose.
7. Other decomposition products of the cerebrosides are ammonia, and a complex material which on fusion with potash yields higher fatty acids.

The cerebrins or cerebrosides.—These substances, the glucoside constitution of which has just been alluded to, form a group of ill-defined, nitrogenous substances, existing especially in the white substance of nervous tissue, and also in the yolk of egg, pus corpuscles, and spleen cells.[2]

[1] *Rep. Med. Off. Privy Council*, London, 1874, p. 113 *et seq.*
[2] Hoppe-Seyler, "Physiol. Chem.," S. 720, 788.

Müller[1] obtained cerebrin by rubbing brain up with baryta water, so as to form a milky fluid; this is boiled, and the resulting coagulum extracted with boiling alcohol; on cooling, the alcoholic solution deposits cerebrin and cholesterin. The latter is removed by ether, and the former is purified by repeated crystallisation from boiling alcohol. According to Müller, its formula is $C_{17}H_{33}NO_3$; according to Parcus,[2] $C_{80}H_{160}N_2O_{15}$. Parcus also obtained two other similar substances (homocerebrin and encephalin) with different formulæ. Adopting a slightly different *modus operandi*, Geoghegan[3] obtained a substance with the formula $C_{37}H_{110}N_2O_{25}$. Thudichum[4] separated three cerebrins, which he named cerebrin ($C_{34}H_{66}N_2O_8$), phrenosine[5] ($C_{34}H_{67}NO_8$), and kerasine ($C_{46}H_{91}NO_9$). Gamgee[6] found that, while protagon cannot be separated by the simple action of solvents into lecithin and cerebrin, yet such non-phosphorised substances do exist by its side in the brain, and one which he called pseudo-cerebrin ($C_{44}H_{92}NO_8$) can be obtained from protagon by the action of caustic baryta.

The fact that the cerebrins are glucosides was known to Liebreich,[7] Diaconow, Otto,[8] Geoghegan,[9] and Thudichum,[10] but it was only within quite recent years that the sugar was identified as galactose, almost simultaneously in this country and in Germany.[11]

The most recent work on the subject is that by Kossel and Freytag,[12] who adopt the very appropriate name of cerebrosides for these bodies. They find that these substances are constituents of the medullary sheaths rather than of the axis cylinders. They have especially worked at two, which they obtained by the decomposition of protagon crystals, namely, cerebrin and kerasin. The analyses of these agree very well with those previously published by Thudichum, Parcus, and others. Their molecular weight was investigated by Beckmann's boiling method, and by the examination of their barium and bromine compounds. By treatment with nitric acid they yield not only galactose but also a fatty acid recognised as neurostearic acid by Thudichum, and correctly analysed but not identified by Müller. It is, in fact, stearic acid, three molecules of which are formed from cerebrin for every two atoms of nitrogen. From all these considerations, the formula given to cerebrin is $C_{70}H_{140}N_2O_{13}$, and to kerasin (the homocerebrin of Parcus), $C_{70}H_{138}N_2O_{12}$.

Similar substances occur in other parts of the body; thus two separated from pus are named *pyosin*, $C_{57}H_{110}N_2O_{15}$, or $C_{58}H_{110}N_2O_{15}$, and *pyogenin*, $C_{63}H_{128}N_2O_{19}$. These bodies and similar ones separated from testicular cells are components of the cell protoplasm, not of the nucleus (Kossel and Freytag).

[1] *Ann. d. Chem.*, Leipzig, Bd. ciii. S. 131; cv. S. 361.
[2] *Journ. f. prakt. Chem.*, Leipzig, Bd. cxxxii. S. 310.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 332.
[4] *Loc. cit.*
[5] For recent papers on phrenosine, see Thudichum, *Journ. f. prakt. Chem.*, Leipzig, Bd. liii. S. 49; Kossel, *ibid.*, 1896, Bd. liv. S. 215.
[6] *Loc. cit.*
[7] *Virchow's Archiv*, Bd. xxxix. S. 183.
[8] *Ibid.*, Bd. xli. S. 272.
[9] Geoghegan stated that the reducing substance had the formula $C_{22}H_{45}O_5$; he termed it cetylid; cetylid was no doubt a mixture of galactose and fatty acids.
[10] *Journ. f. prakt. Chem.*, Leipzig, Bd. xxv. S. 23.
[11] Thierfelder, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 209; Brown and Morris, *Proc. Chem. Soc. London*, 1889, p. 167.
[12] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvii. S. 431.

The Eye.

The cornea.—A thousand parts of corneal tissue contain 242 of solids, of which 204 consist of collagen, 28 of other organic matters, and 10 of ash.[1]

The erroneous idea that the cornea, like cartilage, contains a specific substance called chondrin (Müller), was first combated by Morochowetz,[2] who showed that chondrin here as elsewhere is a mixture of gelatin and a mucinoid material. This latter substance is named by C. T. Mörner,[3] cornea-mucoid; its percentage composition is C, 50·16; H, 6·97; N, 12·79; S, 2·07; O, 28·01. It resembles other mucoids very closely in its properties (see p. 63). The gelatin obtained from the collagen resembles that found elsewhere. The same mucoid and collagen are present in the **sclerotic.**

Descemet's membrane is resistant to reagents. Mörner terms its chief constituent *membranin.* It belongs to the mucoid group. The lens capsule has a similar chemical structure.

The choroid and iris are principally of chemical interest from containing the black pigment which is identical with or nearly related to that in the pigment layer of the retina.

The retina.—Cahn[4] gives the following table of the quantitative composition of the retinæ of geese:—

Water	86 to 89	per cent.
Solids	14 „ 11	„
Proteids (globulin coagulating at 50° C., albumin and mucin (?)) . . .	4 „ 6	„
Gelatin	13 „ 17	„
Cholesterin	0·3 „ 0·8	„
Lecithin	1·0 „ 2·9	„
Fat	0·05 „ 0·5	„
Salts	0·7 „ 1·2	„

The pigments of the retina.—The black pigment of the retinal epithelium is called *fuscin.* In some animals the epithelium is free from pigment in part; this constitutes the *tapetum lucidum.* In some fish this contains crystals of guanine; in the ox and sheep it does not.[5]

Fuscin is one of the group of black pigments, termed *melanins.* It was investigated by Berzelius, and by Heintz, who found it contained a small quantity of iron, by Scherer, who found no iron, and also by Rosow and Sieber. The percentage composition obtained by the various observers shows great discrepancies, and this, taken into account with their methods of preparing the pigment, renders it probable they were dealing with impure substances. The failure to find iron was due to the fact that hydrochloric acid was employed at one stage of the operations, and this dissolves out nine-tenths of the iron.[6]

[1] His, quoted by Gamgee, "Physiological Chemistry," vol. i. p. 451.
[2] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, pt. 5, Bd. i.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 213.
[4] Hoppe-Seyler, "Physiol. Chem.," S. 699.
[5] Kühne and Sewall, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, N. F., Bd. ii. Heft 5.
[6] K. A. H. Mörner, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xi. S. 66. The pigment in the skin of negroes, and in melanotic sarcomata, is closely allied to fuscin. It appears to contain iron. In melanotic sarcomata, Berdez and Nencki named the pigment phymatorusin; in those of horses, hippomelanin. The subject of melanin in the urine has been

May's method[1] of preparing fuscin is to boil retinæ in alcohol, then in ether, lastly in water. The residue is then subjected to tryptic digestion. Three things remain undigested; of these nuclein is got rid of by trituration with alkali; the second, neurokeratin, must be picked out with forceps; the third is the pigment.

Fuscin is slowly bleached in the air; it dissolves by boiling it a long time with concentrated sulphuric acid, or caustic alkalis.

There is a considerable doubt, as in the case of other melanins, such as those in the skin, whether or not it is derived from hæmoglobin.[2] Krükenberg considers it is more closely related to the lipochromes. It is, however, undoubtedly nitrogenous. It is certainly not a member of the group of pigments occurring in plants named humous substances by Hoppe-Seyler,[3] since on fusing with alkali it yields no pyrocatechin or protocatechnic acid.[4] The chief interest of fuscin is not, however, chemical but physiological. Such problems as its varying distribution under the influence of light and its relationship to the visual purple of the rods will be treated under "Vision."

Visual purple or rhodopsin.—We possess very little chemical knowledge of visual purple. Kühne found it to be soluble in certain reagents such as solutions of bile salts, that in the process of bleaching it passes through a yellow stage, that the bleaching occurs at different rates at different temperatures and in different coloured lights, and that spectroscopically it cuts out a very considerable portion of the spectrum. It is destroyed by alcohol, ether, chloroform, and strong alkalis and acids, but not by most oxidising agents. It is perhaps related to the lipochromes. The green, yellow, and red pigments (*chromophanes*) of the oil droplets in the cones of birds are undoubtedly lipochromes (see p. 20).

The aqueous humour is lymph.[5] In parts per 1000 it contains: water, 986·87; solids, 13·13; proteids, 1·22; extractives, 4·21; inorganic salts, 7·70; sodium chloride, 6·89.[6] It does not clot spontaneously, but does so on addition of serum. The proteids in it are fibrinogen, serum globulin, and serum albumin.[7] Kühne[8] found a reducing substance among the extractives. This is not sugar. Urea and sarcolactic acid are also present in small quantities.[9]

The vitreous humour.—The membranes of the vitreous humour

worked at especially by v. Jaksch. The following are references to the principal papers on the subject:—Mörner, *loc. cit.*, also *ibid.*, Bd. xii. S. 229; Nencki, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxiv. S. 17, 27; *Chem. Centr.-Bl.*, Leipzig. 1888, S. 587; Brandl and Pfeiffer, *Ztschr. f. Biol.*, München, Bd. xxvi. S. 348; v. Jaksch, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii.; Abel and Davis, *Journ. Exper. Med.*, Baltimore, 1896, No. 3, vol. i.; Schmiedeberg, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1897, Bd. xxxix. S. 1.

[1] *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, Bd. ii. S. 324.

[2] Delépine has even suggested that, in the case of the skin pigment, hæmoglobin is derived from it (*Proc. Physiol. Soc.*, London, Dec. 13, 1890, p. xxvii.). Abel and Davies (*loc. cit.*) have recently studied the pigment of the negro's skin. The granules contain inorganic matter as well as pigment. The latter contains the merest trace of iron. They conclude that it originates not from hæmoglobin, but from the proteids of the tissue juice.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 66.

[4] Hirschfeld, *ibid.*, Bd. xiii. S. 407.

[5] Chavvas, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xvi. S. 143.

[6] Lohmeyer. See Gorup-Besanez, "Lehrbuch," 4th edition, 1878, S. 401.

[7] Friend and Halliburton, *Rep. Brit. Ass. Adv. Sc.*, London, 1889, p. 130.

[8] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xii. S. 200.

[9] Grünhagen, *ibid.*, Bd. xliii. S. 377; Pautz, *Ztschr. f. Biol.*, München, Bd. xxxi.

yield gelatin. Its chief constituent is mucin, or mucinogen (Young), called mucoid by C. T. Mörner. According to the latter, this mucoid contains 12·27 nitrogen, and 1·19 sulphur, per cent. There are also small quantities of proteid. References to the papers of Young and Mörner, the most recent workers on this subject, will be found on p. 62.

The lens.—The following are the results of Laptschinsky's[1] analyses:—

Water .	63·50 per cent.	Cholesterin[2] .	0·22 per cent.
Solids .	36·50 „	Fats .	0·29 „
Proteids	34·93 „	Salts .	0·82 „
Lecithin	0·23 „		

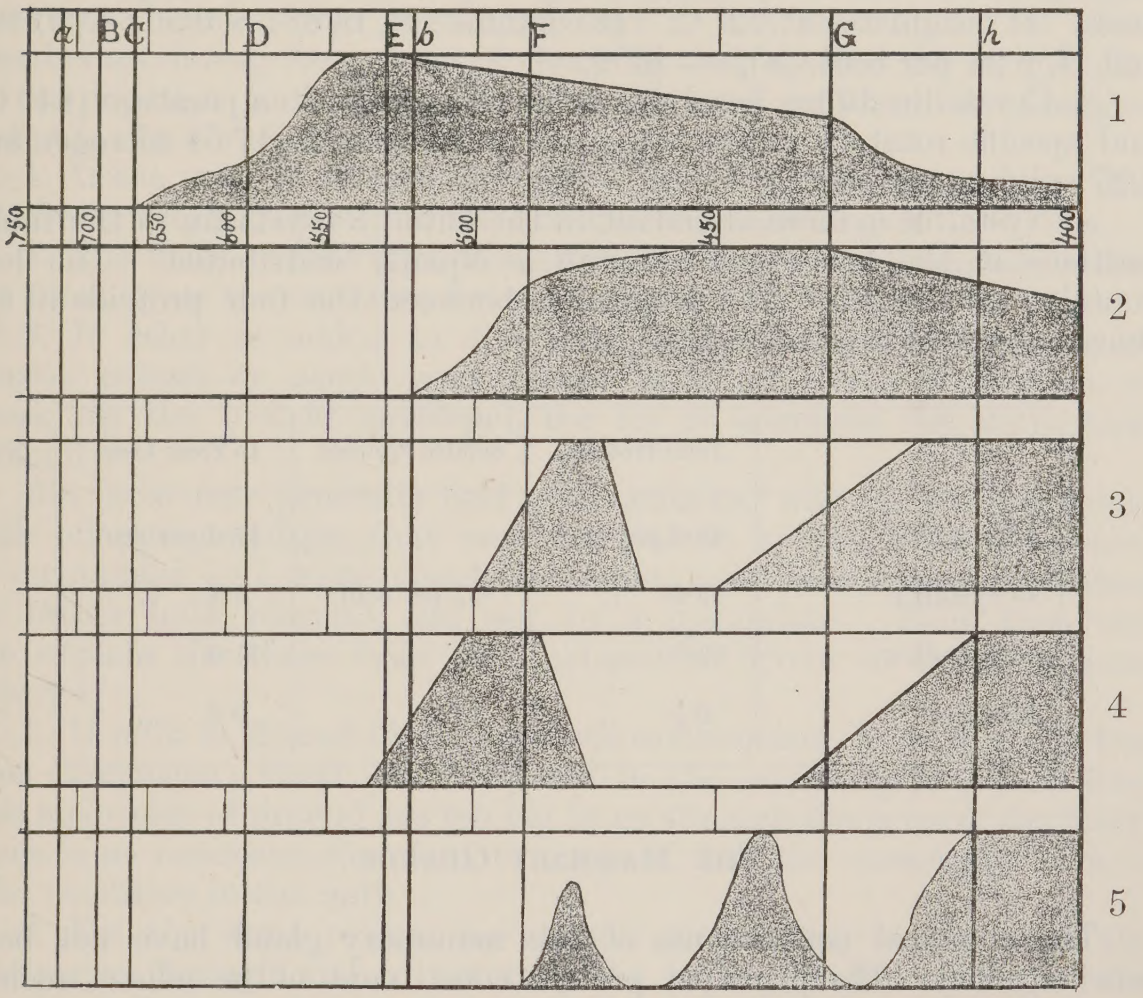


FIG. 20.—Absorption spectra of retinal pigments.—1, of visual purple; 2, of visual yellow; 3, of xanthophane in ether; 4, of rhodophane in turpentine; 5, of chlorophane in ether. This diagrammatic way of representing absorption spectra indicates the thickness of the absorption-bands in solutions of different strengths; the top of each spectrum shows the thickness of the bands in a dilute solution; as the concentration of the solution increases, the bands become wider, as in the lower part of each diagram.—After Kühne.

The proteid matter is thus very abundant; it is chiefly a globulin, to which Berzelius gave the name of crystallin. It has also been the subject of researches by Hoppe-Seyler, Laptschinsky, Kühne, and C. T. Mörner.[3] According to the last-named investigator, about 52 per cent. of the proteid matter of the lens is insoluble in water and saline solutions. The insoluble proteid residue is an *albuminoid*, and it is

[1] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xiii. S. 631.

[2] The cholesterin increases greatly in cataract (Cahn, Hoppe-Seyler's "Physiol. Chem.," S. 692).

[3] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xviii. S. 61.

most abundant in the inner denser portions of the lens. It yields no nuclein on gastric digestion; the small amount of phosphorus it contains is due to inorganic phosphates. The soluble proteids of the lens are also not nuclein compounds. About one per cent. of the soluble proteid is albumin; the rest is globulin. The globulin is precipitated by saturation with magnesium sulphate, but not with sodium chloride; in this it resembles vitellin. The globulin consists of two proteids, α–crystallin and β–crystallin.

α–Crystallin is completely precipitable by saturation with magnesium sulphate or with sodium sulphate at 30° C., by the addition of one and a half times its volume of saturated ammonium sulphate solution, by a stream of carbonic anhydride, and by very dilute acetic or hydrochloric acids. It coagulates at 72° C. It contains: N, 16·68; S, 0·56; C, 52·83; and H, 6·94 per cent. $(\alpha)_D = -46°·9$.

β–Crystallin differs from this in its coagulation temperature (64° C.) and specific rotatory power $(\alpha)_D = -43°$. It contains 17·04 nitrogen and 1·27 sulphur per cent.

α-Crystallin is more abundant in the outer, β-crystallin in the inner, portions of the lens; the albumin is equally distributed. The lens contains no keratin. The proportion between the four proteids in the lens as a whole is as follows:—

	Total Proteids.	Soluble Proteids.	In Fresh Lens.
Albuminoid . .	48·0 per cent.	...	17·0 per cent.
α-Crystallin . .	19·5 ,,	37 per cent.	6·8 ,,
β-Crystallin . .	32·0 ,,	62 ,,	11·0 ,,
Albumin . .	0·5 ,,	1 ,,	0·2 ,,

The Mammary Glands.

The chemical constituents of the mammary gland have not been much studied. The principal proteid constituent of the cells is nucleo-proteid, which swells with dilute alkali, and yields, by boiling with mineral acid, a reducing substance. That a reducing substance (sugar) can be obtained from the gland was first noted by Bert,[1] and confirmed by Landwehr,[2] who considered its mother substance to be animal-gum; it is considered by Thierfelder[3] to be the mother-substance of lactose. It is possible that the nucleo-proteid just mentioned may be the precursor of caseinogen. The lactalbumin of milk is not identical with serum albumin, so that its presence in milk cannot be explained by a simple transudation from the blood.

The extractives of the mammary gland contain not unimportant quantities of hypoxanthine;[4] they have not been further investigated.

[1] *Gaz. hebd. de méd.*, Paris, 1879, No. 2; *Compt. rend. Acad. d. sc.*, Paris, tome xcviii.

[2] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xl. S. 21. Thierfelder had previously (*ibid.*, Bd. xxxii. S. 619) recognised that the substance is not glycogen.

[3] *Loc. cit.*

[4] Hammarsten, "Physiol. Chem.," S. 378.

Milk.

General properties and composition.—Milk consists of fluid (milk plasma) in which are suspended innumerable minute globules of fat.[1] It is therefore an emulsion, and its white colour is produced, as in other emulsions, by reflection from the surface of the numerous globules. The specific gravity of cow's and of human milk is about the same, namely, 1028 to 1034.[2] It is increased by the removal of the lightest constituent, the cream. Among the milk globules are smaller particles of proteid matter (caseinogen or nuclein ?).[3]

The statement is still often made that each fat globule in milk is surrounded by a thin membrane of caseinogen—the so-called haptogen membrane,[4] and it was considered that it was the rupture of these membranes during the process of churning that enabled the fat globules to run together to form butter. The evidence on which this idea has rested is of a threefold nature :—

1. If the milk is filtered through a cell of porous earthenware, the filtrate is free, not only from fat, but also from caseinogen.

2. The mass of milk globules, after having been well washed within the filter, gives the reactions for caseinogen.[5]

3. If ether is added to the milk, without previous addition of caustic potash or acetic acid (these were supposed to dissolve or break up the proteid envelope), the fat is dissolved out with great difficulty.

But it is now generally held with Quincke,[6] who made experiments with oil and mucilage, that each fat globule by molecular attraction is surrounded by a more closely adherent layer of caseinogen solution (or rather milk plasma), and not by a membrane. How then can one explain the three facts just adduced in favour of the membrane theory ?

1. If milk is filtered through porous earthenware, it is naturally free from caseinogen; blood serum filtered in the same way is proteid free. The molecules of proteid are too big to go through the pores of the filter; there is no necessity, therefore, to suppose that the caseinogen is in a solid condition in the milk.

2. For the same reason, no amount of washing would wash the caseinogen through, and so naturally the milk globules would give the reactions of the proteid with which they are contaminated. Further, Hoppe-Seyler[7] has shown that cream yields the same percentage of casein as the layers of milk below it.

3. The addition of reagents such as acetic acid (and rennet) enables the fat to pass into solution more easily, not because they are solvents of proteid, for they are not, but because they alter the relations between the surface tensions of fat globules and milk plasma, and so

[1] For the measurement and examination of the fat globules, see Fleischmann, "Das Molkereiwesen," Braunschweig, 1876–9, S. 206; F. W. Woll, "Wisconsin Exper. Stat. Agric. Sc.," 1892, vol. vi.

[2] For observations on the specific gravity of human milk, see Monti, *Arch. f. Kinderh.*, Stuttgart, Bd. xiii.

[3] Kehrer, *Arch. f. Gynaek.*, Berlin, Bd. ii. S. 1; D. F. Harris, *Proc. Roy. Soc. Edin.*, 1896, p. 72.

[4] Ascherson, *Arch. f. Anat. u. Physiol.*, Leipzig, 1840, S. 53.

[5] Radenhausen and Danilewsky, *Forsch. a. d. Geb. d. Viehhaltung*, Bremen, 1880, Heft 9.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix. S. 129.

[7] "Physiol. Chem.," S. 728.

enable the ether to attack the fat more easily. Moreover, Hoppe-Seyler states that it is not so difficult to remove the fat simply with ether; the fluid still remains cloudy, it is true, but solutions of caseinogen are always opalescent, and this is increased by the presence in the milk of particles of proteid or proteid-like substances, as described by Kehrer.

The reaction of milk.—Milk readily turns sour from the fermentation of lactose and formation of lactic acid. In carnivora fresh milk has an acid reaction, but in most animals it gives either an alkaline or, more frequently, an amphoteric reaction; the acid phosphates in the milk turn neutral litmus red, and the alkaline phosphates turn it blue. The proportion between these salts varies very considerably in different animals, in the same animal at different stages of lactation, and even between the first and last portions of the same milking (Thörner,[1] Sebelien,[2] Courant[3]).

Courant estimated the alkaline constituent by titration with decinormal sulphuric acid, with blue lackmoid as indicator, and the acid constituent with decinormal soda with phenolphthaleïn as indicator. He found as a mean for the first and last portions of the milking of twenty cows, that the alkalinity of 100 c.c. of the milk was equal to 41 c.c., and the acidity equal to 19·5 c.c. of the respective solutions used. In human milk the proportional alkalinity is higher; the average of the numbers was 10·8 and 3·6 c.c. respectively.

Constituents of milk.—These are water, three proteids (caseinogen, lactalbumin, lacto-globulin), two carbohydrates (lactose, animal gum ?), fats, extractives (traces of urea, creatine, creatinine, hypoxanthine, lecithin, cholesterin, citric acid[4]), salts and gases. Most of these demand separate discussion.

Effect of boiling milk.—When milk is heated to, or near to, the boiling point, a scum forms on the surface; on the removal of this skin it is rapidly renewed, and this can be repeated over and over again. This is probably in part produced by the coagulation of the lact-albumin; this carries to the surface some caseinogen and fat.[5] Contact with air appears to be the chief influence in causing the solidification which results in the formation of the scum; evaporation is rapid from the surface exposed to the atmosphere, and thus partial drying occurs there.

The boiling of milk before it is used as a food is advantageous in two ways—(1) all micro-organisms are destroyed; (2) the gastric juice, in virtue of its rennet, causes a flocculent and not a bulky precipitate. These quite outweigh any slight diminution of digestibility alleged to occur.[6] The reason that boiled milk curdles with rennet with greater difficulty than fresh milk appears to be that, by boiling, a part of the dissolved calcium salt is precipitated as tricalcium phosphate.

As milk turns sour, it is possible to get a bulky heat coagulum by boiling.[7]

[1] *Chem. Ztg.*, Cöthen, Bd. xvi. S. 1469. [2] *Ibid.*, S. 597.
[3] Inaug. Diss., Bonn, 1891; and *Arch. f. d. ges. Physiol.*, Bonn, Bd. l.
[4] Söldner, *Landw. Versuchs. Stat.*, Berlin, Bd. xxxv.
[5] See D. F. Harris, *Journ. Anat. and Physiol.*, London, 1894, vol. xxix. p. 188.
[6] Raudnitz, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 1.
[7] Recent work on this question will be found in a paper by Cazeneuve and Haddon, *Compt. rend. Acad. d. sc.*, Paris, 1895, tome cxx. p. 1272. See also influence of boiling on the proteids of cows' milk, *Centralbl. f. d. med. Wissensch.*, Berlin, Bd. xxxiv. S. 145.

Under the influence of extracts of the pancreas, the caseinogen, before it is clotted by the milk-curdling ferment of the gland, passes through a stage in which it coagulates by heat. This was termed the "metacasein" reaction by its discoverer, Sir William Roberts.[1] It does not appear to be due to the simultaneous development of acid produced by the fat-splitting ferment of the pancreas, but rather to the action of trypsin. Edkins[2] showed that Kühne's purified trypsin also produces "metacasein" in an early stage of its action, though it does not produce coagulation of milk.

The composition of milk varies in different animals; human milk and cows' milk are those which have been most investigated. There are also variations due to constitution, state of nutrition, and age.

Human milk.—The mammary glands of new-born animals of both sexes often secrete a small quantity of milk for a few days. It is popularly termed "*witches' milk.*" It is alkaline.[3] Analyses by Schlossberger and Hauff,[4] Gubler and Quevenne,[5] and Genser,[6] show that the milk of new-born children contains from 1·05 to 2·8 proteid, 0·82 to 1·46 fat, 0·9 to 6·4 sugar, and 0·8 salts per cent.

Colostrum.—This liquid is yellower and more alkaline than fully-formed milk. It contains colostrum corpuscles, rather more solids than milk, and coagulates on heating. It contains little or no caseinogen, but a mixture of lacto-globulin and lactalbumin.[7] The globulin is only present in traces in fully-formed milk. The following analyses are by Clemm,[8] with the exception of the last, which is by Tidy.[9]

Constituents.	Four Weeks before Delivery.		Seventeen Days before Delivery.	Nine Days before Delivery.	Twenty-four Hours after Delivery.	Two Days after Delivery.	
	I.	II.					
Water .	94·52	85·2	85·17	85·85	84·38	86·79	84·08
Solids . .	5·48	14·8	14·83	14·15	15·62	13·21	15·92
Casein .	...	...	...	...	...	2·18	
Albumin and globulin	2·88	6·9	7·48	8·07	...	...	3·23
Fat . .	0·71	4·1	3·02	2·35	...	4·86	5·78
Lactose .	1·73	3·9	4·37	3·64	...	6·10	6·51
Salts . .	0·44	0·44	0·45	0·54	0·51	...	0·34

[1] *Proc. Roy. Soc. London*, 1879, 1891.

[2] *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 203.

[3] Witches' milk obtained from foals by Ammon (*Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, S. 118) was acid, but this was probably due to fermentation having set in.

[4] *Ann. d. Chem.*, Leipzig, Bd. xcvi. S. 68.

[5] *Gaz. méd. de Paris*, 1856, p. 15.

[6] *Jahrb. f. Kinderh.*, Leipzig, N. F., Bd. ix. S. 60.

[7] J. Sebelien, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 135.

[8] Wagner's "Handwörterbuch d. Physiol," Bd. ii. S. 464.

[9] *Lond. Hosp. Rep.*, 1867–8, p. 77. See also Woodward (*Journ. Exper. Med.*, Baltimore, 1897, vol. ii. p. 217), for recent analyses of human colostrum. Colostrum corpuscles are not constantly present.

Normal human milk.—The following table gives some of the principal analyses that have been published:—

Water.	Caseinogen.	Albumin.	Fat.	Sugar.	Salts.	Remarks.	Observers.
88·58	3·69		3·53	4·3	0·17	9 days after delivery.	Clemm.
90·58	2·91		3·34	3·15	0·19	12 ,, ,, ,,	Clemm.
86·27	2·95		5·37	5·13	0·22	.....	Tidy.
86·3 to 88·8	1·68 to 3·15		2·6 to 5·4	5·8 to 6·6	0·23 to 0·34	.....	Biel.[1]
...	1·28	0·34	2·56	5·6	...	...	Tolmatscheff.[2]
89·1	1·79		3·3	5·4	0·42	...	Gerber.[3]
87·24	1·9		4·3	5·9	0·28	...	Christenn.[4]
89·29	1·6		3·2	5·8	0·16	Women 20-30 years old	Pfeiffer.[5]
89·06	1·72		2·9	6·0	0·2	,, 30-40 ,,	Pfeiffer.[5]
87·79	2·53		3·9	5·5	0·25	...	Mendus de Leon.[6]
...	1·8 to 4·8	0·7 to 1·7	...	...	...	...	Makris.[7]
97·6	1·52		3·28	6·50	0·27	...	Söldner & Camerer.[8]
88·5	1·2	0·5	3·3	6·0	0·2	...	Lehmann & Hempel.[9]

The most constant feature in these analyses is the relatively low percentage of proteids and high percentage of sugar.

Among other constituents of human milk are, 0·32 per cent. of cholesterin (Tolmatscheff), 0·05 of citric acid,[10] and 0·78[11] of unknown extractives; the last are more abundant in the colostrum, and less abundant in cows' milk (Söldner and Camerer).

Variations in the composition of the milk occur with the stages of lactation,[12] in the milk from the two breasts and between the first and last portions of the milking,[13] with the complexion [14] (Vernois and Becquerel—questioned by Tolmatscheff), with the age of the individual (Pfeiffer), and with menstruation (Vernois and Becquerel). The nature and quality of the food have a considerable influence on the quality of the milk.[15]

The salts of human milk are thus given by Bunge [16] in parts per 1000.—

	A.	B.		A.	B.
K_2O . .	0·780	0·703	Fe_2O_3 . .	0·004	0·006
Na_2O . .	0·232	0·257	P_2O_5 . .	0·473	0·469
CaO . .	0·328	0·343	Cl . . .	0·438	0·445
MgO . .	0·064	0·065			

[1] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, S. 168.
[2] "Med. Chem. Untersuch.," Bd. ii. S. 272.
[3] *Bull. soc. chim.*, Paris, tome xxiii. [4] Diss., Erlangen, 1877.
[5] *Jahrb. f. Kinderh.*, Leipzig, Bd. xx. [6] Diss., Heidelberg, 1881.
[7] Diss., Strassburg, 1876. [8] *Ztschr. f. Biol.*, München, 1896, Bd. xxxiii. S. 43.
[9] *Arch. f. d. ges. Physiol.*, Bonn, Bd. lvi. S. 558.
[10] The presence of citric acid has also been noticed by Scheibe, *Landw. Versuchs. Stat.*, Berlin, Bd. xxxix.
[11] J. Munk (*Virchow's Archiv*, Bd. cxxxiv. S. 501) gives the proportion of extractive nitrogen to total nitrogen as 1:11 in human and 1:16 in cows' milk.
[12] Pfeiffer, *loc. cit.*; Vernois and Becquerel, *Compt. rend. Acad. d. sc.*, Paris, tome xxxvi. p. 188.
[13] Sourdat, *ibid.*, tome lxxi.; Brummer, *Arch. f. d. ges. Physiol.*, Bonn, Bd. vii.
[14] l'Heritier, "Traité de chim. pathol.," Paris, 1842.
[15] Decaisne, *Gaz. méd. de Paris*, 1871, p. 317. These are very interesting observations made during the siege of Paris. Other work on the influence of food on milk is that by Szubotin, *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, S. 337, and by Commaille, quoted by König, "Chem. d. menschl. Nährungs. u. Genussmittel," Bd. ii. S. 235. The question of the influence of diseases and drugs will be found discussed in works on Therapeutics and Pathology.
[16] *Ztschr. f. Biol.*, München, Bd. x.

The gases of human milk.—In five experiments, 100 c.c. of milk yielded 1·07 to 1·44 c.c. of oxygen, 2·35 to 2·87 c.c. of carbonic anhydride, and 3·37 and 3·81 c.c. of nitrogen. The method of collecting the milk could not have obviated admixture with small quantities of air; hence, no doubt, the higher percentage of oxygen and nitrogen than previous observers have found in the milk of lower animals.[1]

Cows' milk.—*Colostrum.*—This has a high specific gravity (1046–1080). Its fat has a higher melting point than that of normal milk, being poorer in the lower fatty acids.[2] It contains more lecithin, cholesterin, and proteid coagulable by heat than normal milk.[3] The following are some analyses that have been made:—

	Fleischmann.[4]	König.[5]	Vaudin.[6] Just after delivery.	Vaudin.[6] Five days after delivery.
Water	78·7	74·7	77·6–72·7	85·63
Solids	21·3	25·3	22·4–27·4	14·37
Casein	7·3	4·04	14·9–20·1	4·35
Albumin and globulin	7·5	13·6		
Fat	4·0	3·6	2·42–6·3	5·18
Lactose	1·5	2·7	1·02–2·86	4·07
Salts	1·0	1·6	1·1–1·2	0·16

Normal cows' milk.—The following are averages of numerous analyses, in the first column of those collected by Gorup-Besanez,[7] in the second, by Hoppe-Seyler.[8]

	I.	II.
Water	84·28	85–86
Solids	15·72	15–18
Caseinogen	3·57	3–4
Albumin	0·75	0·3–0·5
Fat	6·47	4
Lactose	4·34	4·5–5
Salts	0·63	...

Hammarsten[9] gives the following tables (from König) of normal milk and the averages of various preparations from milk as follows:—

	Milk.	Skimmed Milk.	Cream.	Butter Milk.	Whey.
Water	87·17	90·66	65·51	90·27	93·24
Solids	12·83	9·34	34·49	9·73	6·76
Caseinogen	3·02	3·11	3·61	4·06	0·85
Albumin	0·53				
Fat	3·69	0·74	26·75	0·93	0·23
Sugar	4·88	4·75	3·52	3·73	4·7
Salts	0·71	0·74	0·61	0·67	0·65
Lactic acid	...	...	...	0·34	0·33

Tatlock[10] gives the average composition of skimmed milk as follows—(1)

[1] E. Külz, *Ztschr. f. Biol.*, München, 1895, Bd. xxxii. S. 180.
[2] Nilson, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xvii. S. 169.
[3] *Ibid.*, Bd. xviii. S. 102.
[4] "Das Molkereiwesen," S. 39. [5] "Chem. d. Mensch. Nährungsmittel."
[6] *Journ. de pharm. et chim.*, Paris, 1894, Sér. 5, tome xxx. p. 337.
[7] "Lehrbuch," 1878, S. 424. [8] "Physiol. Chem."
[9] "Physiol. Chem.," 3rd German edition, S. 388, 390.
[10] "Produce of the Dairy," Glasgow, 1888. Numerous milk analyses will be found in this book.

By repose and skimming—fat, 1; proteid, 3·44; lactose, 5·1; ash, 0·75; water, 89·67. (2) By separator—fat, 0·2; proteids, 3·4; lactose, 5·01; ash, 0·75; and water, 90·64.

The presence of citric acid in milk was first shown by Soxhlet. Vaudin[1] considers that this is not from the food, but produced in the mammary gland.

The variations in the milk with feeding, species of animal, time of day, etc., are described by Struckmann and Bödeker,[2] Fleischmann,[3] Tatlock,[3] Kühne and Fleischer,[4] and others.

Salts of cows' milk.—Söldner[5] gives the following percentages:—

K_2O	0·172
Na_2O	0·051
CaO	0·198
MgO	0·020
P_2O_5 (after correction for pseudo-nuclein)	0·182
Cl	0·098

Of the total phosphoric acid, from 36 to 56 per cent., and of the lime from 53 to 72 per cent., is not simply dissolved in the fluid, but is united more or less firmly to the caseinogen. The excess of bases over mineral acids is united to organic acids, such as citric. Bunge found 0·00035 per cent. of iron.

The gases of cows' milk have been analysed by Setchenow[6] and Pflüger.[7] There are small quantities of oxygen and nitrogen, and from 5 to 10 per cent. of carbonic anhydride.

In comparing the composition of cows' milk with that of human milk, the main difference consists in the high percentage of proteids, fats, and salts, and the low percentage of sugar in cows' milk as compared with human milk. Qualitative differences will be noted under the headings "Proteids" and "Fats."

The milk of other animals.—Some of the principal analyses are collected into the following table:—

Animal.	Water.	Caseinogen.	Albumin.	Fat.	Lactose.	Salts.	Reference to Notes below.
Dog	75·4	9·91		9·57	3·19	0·73	8
Cat	81·6	9·08		3·33	4·91	0·58	8
Goat	86·91	3·69		4·09	4·45	0·86	9
Goat	86·75	3·64		5·35	3·60	0·66	10
Sheep	83·5	5·74		6·14	3·96	0·66	11
Sheep	82–84	4·7		4–8	3–4·6	0·6	12

[1] *Journ. de pharm. et chim.*, Paris, tome xxx. p. 464.
[2] *Ann. d. Chem.*, Leipzig, Bd. xcvii. S. 150.
[3] *Loc. cit.*
[4] *Landw. Versuchs. Stat.*, Berlin, Bd. xii. S. 405.
[5] *Loc. cit.*
[6] *Ztschr. f. rat. Med.*, Bd. x. S. 285.
[7] *Arch. f. d. ges. Physiol.*, Bonn, Bd. ii. S. 166.
[8] Taken from König's analyses. The milk is acid, rich in proteids and calcium. The lactose is increased by starchy, and lessened though not abolished by a flesh diet (Bensch, *Ann. d. Chem.*, Leipzig, Bd. lxi. S. 221; Poggiale, *Gaz. méd. de Paris*, Sér. 3, tome x. p. 259). See also Simon, "Die Frauenmilch," Berlin, 1838; Dumas, *Compt. rend. Acad. d. sc.*, Paris, tome xxi. p. 707; Kemmerich, *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, No. 30; Szubotin, *ibid.*, No. 22.
[9] Taken from König.
[10] From Pizzi's analyses (*Staz. Sper. Agrar.*, 1894, Bd. xxvi. S. 615; Abstract in *Journ. Chem. Soc.*, London, 1896, vol. ii. p. 120). Goats' milk differs from cows' milk in smell and taste, and in containing more insoluble volatile fatty acids. In reindeer's milk these acids are less abundant (Solberg, *Centralbl. f. agric. Chem.*, Leipzig, 1896, S. 15).
[11] From König.
[12] From Vernois and Becquerel, *Union méd.*, Paris, 1867, p. 78.

The milk of other animals—*continued.*

Animal.	Water.	Caseinogen.	Albumin.	Fat.	Lactose.	Salts.	Reference to Notes below.
Sheep . .	80·42	4·44		9·66	4·4	1·1	1
Mare . .	90·06	1·89		1·09	6·65	0·31	2
,, . .	90	1·8	0·3	1·3	5·5	0·3	3
,, . .	92·5	1·3	0·3	0·6	4·7	0·3	4
,, . .	91·0	1·05		1·3	5·7	0·3	5
Ass . . .	90·0	2·1		1·3	6·3	0·3	6
Ass . . .	90·5	1·7		1·4	6·40		7
Ass . . .	89·0	3·5		1·8	5·0	0·5	8
Pig . . .	82·37	6·09		6·4	4·04	1·06	9
Pig . . .	83·0	7·0		7·0	2.0	1·05	10
Pig . . .	81·8	5·3		6·0	6·0	0·08	10
Mule . . .	89·3	2·6		1·9	6·03	0·53	11
Hippopotamus .	90·0	...		4·5	...	0·1	12
Camel . .	86·3	3·7		2·9	5·8	0·6	13
Elephant . .	67·85	3·09		1·95	8·84	0·65	14
Dolphin . .	48·67	...		43·76	...	0·46	15
Buffalo . .	82·20	4·13		7·95	4·75	0·97	16
Rabbit . .	69·50	15·54		10·4	1·95	2·56	17

Salts of dogs' and mares' milk.—These may be compared in the following table of Bunge's [18] with those we have already studied :—

	Human.		Dog.		Cow.	Mare.
	I.	II.	I.	II.		
K_2O	0·78	0·71	1·41	1·68	1·76	1·04
Na_2O	0·23	0·26	0·80	0·69	1·11	0·14
CaO	0·33	0·34	4·53	4·28	1·59	1·23
MgO	0·06	0·06	0·19	0·21	0·21	0·12
Fe_2O_3	0·003	0·006	0·02	0·01	0·003	0·015
P_2O_5	0·47	0·47	4·93	4·67	1·97	1·31
Cl	0·43	0·44	1·62	1·8	1·69	0·31
Total ash per 1000 . . .	2·22	2·18	13·15	12·96	7·97	4·17

[1] From Pizzi. Sheep's milk contains a high percentage of fat.
[2] From König.
[3] From Biel, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1864, S. 171.
[4] From Soxhlet, *ibid.*, 1878, S. 152.
[5] From Weiske and Schrodt, *ibid.*, 1878, S. 151. The caseinogen of mares' milk is more like that of human than of cows' milk. This is the milk originally used in the preparation of koumiss and similar fermented liquors in Russia.
[6] From König.
[7] From Gubler and Quevenne, Gmelin's "Handbuch," Bd. viii. S. 267.
[8] From Vernois and Becquerel. Asses' milk is much used by invalids.
[9] From König.
[10] From Leutner, in Gorup-Besanez, "Lehrbuch," S. 424.
[11] From Aubert and Colby, *Chem. News*, London, vol. lxviii. p. 168.
[12] From *Chem. Centr.-Bl.*, Leipzig, 1871, S. 149.
[13] From Dragendorf, *ibid.*, 1867, S. 78. See also Vernois and Becquerel for analyses of camels' milk.
[14] From König.
[15] From Frankland, *Chem. News*, London, 1890, vol. lxi. Note the enormous percentage of fat.
[16] From Pizzi. For buffaloes' milk, see also Pappel and Richmond, *Journ. Chem. Soc.*, London, 1894, p. 754.
[17] From Pizzi.
[18] Diss., Dorpat., 1874 ; *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 399.

The chief acid present throughout is phosphoric acid; the chief base in human milk is potash; but this in most other mammals is second to lime; in dogs' milk the lime is especially high.

In connection with the iron in the milk it is to be noticed, that although the other mineral constituents of the milk are present in the proportion in which they are contained in the fœtal tissues, the quantity of iron in milk is less.

One hundred parts by weight of ash contain—

	In New-born Dog.	In Dogs' Milk.
K_2O	11·42	14·98
Na_2O	10·64	8·80
CaO	29·52	27·24
MgO	1·82	1·54
Fe_2O_3	0·72	0·12
P_2O_5	39·42	34·22
Cl	8·35	16·90

The slightly different proportion in soda and potash is easily explained by the fact that in the young animal the potash-rich muscle is increasing, and the soda-rich cartilage is diminishing. The high percentage of chlorine in the milk is also explicable, on the hypothesis that the chlorides serve not only to build up tissues, but also largely as solvents in removing waste products. But the percentage of iron in the milk is only one-sixth of that in the fœtal tissues. The fœtus obtains its supply of iron before birth through the placenta, and stores it in the liver (see p. 86). As the young animal grows, a kilogram of body weight contains less and less iron.

Iron appears to pass to the offspring through the placenta rather than by the milk, because of the difficulties of absorbing iron by the alimentary canal, and the danger that hæmatogenous (*i.e.* nuclein) compounds may there become the prey of bacteria. Bunge further regards it as probable that the large amount of iron which passes to the fœtus is not all derived from the mother's food during the relatively short period of pregnancy, but that a storage of iron occurs in the maternal organs even before the first conception; and this may explain the occurrence of chlorosis at the age of puberty.

The carbohydrates of milk.—The most important carbohydrate in milk is lactose, or milk-sugar, the properties of which are described on page 12. It is found in varying quantities in the milk of all animals; the only exception to this rule hitherto noted is that of the Egyptian buffalo (*Bos bubalus*), where it is replaced by another sugar christened *tewfikose*[1] by Pappel and Richmond;[2] it yields dextrose only on hydrolysis.

Though lactose is not fermented by yeast, yet it undergoes the alcoholic fermentation under the influence of other schizomycetes, as in the preparation of koumiss and kephir.

Ritthausen[3] found in milk another carbohydrate which is soluble in water, and is not crystallisable; its reducing power is low, and increased after boiling

[1] After the Khedive of Egypt.
[2] *Journ. Chem. Soc.*, London, 1894, p. 754.
[3] *Journ. f. prakt. Chem.*, Leipzig, N. F., Bd. xv.

with acid. Landwehr[1] identified it as animal gum, Béchamp[2] as dextrin. J. Herz[3] found granules in milk, which behave towards iodine like starch; he called them "animal amyloid."

The fats of milk.—Milk fat has a specific gravity of from 949 to 996.[4] It consists of palmitin, stearin, and olein, with small quantities of triglycerides of butyric, caproic, caprylic, capric, myristic, and arachic acids in addition.[5] It also contains small quantities of lecithin, cholesterin, and a yellow lipochrome.

The amount of fat in cream varies from 14 to 44 per cent. In butter, besides fat, there are small quantities of caseinogen and lactose. The fats of cows' butter consist of 68 per cent. of palmitin and stearin, 30 per cent. of olein, and 2 per cent. of the specific butter fats.[6] Their melting point is 31° to 34° C. The volatile fatty acids in cows' milk, according to Duclaux,[7] amount to 7 per cent., of which 3·7 to 5·1 is butyric, and 2·0 to 3·3 is caproic acid. Some analysts give still higher percentages.

By exposure to the air butter becomes rancid; this is partly due to the production of lower fatty acids from the higher fats (see p. 19), partly to the formation of acrolein from glycerine, and partly, and according to Hagemann chiefly, to the formation of lactic acid from the entangled lactose.

The composition of butter is very variable. Thus, in Finland butter, Koefoed[8] found two fatty acids of the acrylic series in addition to oleic acid; 100 parts of the fatty acid contained 66 of these acids, 28 of palmitic, 22 of myristic, 8 of lauric, 1·5 of butyric, 2 of caproic, 2 of capric, and 0·5 of caprylic acid. According to Wanklyn,[9] there is no true palmitic acid in butter; the acid is aldepalmitic acid ($C_{16}H_{30}O_2$).

The fats of human milk are somewhat different from those of cows' milk. They have been the subject of two recent researches—one by Ruppel,[10] the other by Laves.[11]

Their melting point is 34° C., and solidifying point 20°·2 C. Their specific gravity at 15° C. is 966. The fatty acids found are butyric, caproic, capric, myristic, palmitic, stearic, and oleic acids, all combined with glycerine. The presence of formic acid[12] is also inferred from its reducing action, but not by any further tests. Human milk is poor in volatile acids (Ruppel).

Laves confirms this work, and gives some quantitative results. The fat contains 1·4 per cent. of volatile acids, 1·9 of acids soluble in water, and 49·4 (a very high percentage) of unsaturated acids. The volatile

[1] *Arch. f. d. ges. Physiol.*, Bonn, Bde. xxxix. and xl.
[2] *Bull. Soc. chim.*, Paris, Sér. 3, tome vi.
[3] *Chem. Ztg.*, Cöthen, Bd. xvi. S. 1594.
[4] Bohr, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. x. S. 182.
[5] Grunzweig, *Ann. d. Chem.*, Leipzig, Bd. clxii. S. 215; E. Wein, Diss., Erlangen, 1876; Chevreul, "Recherches sur le corps gras," Paris, 1823; Lerch, *Ann. d. Chem.*, Leipzig, Bd. xlix. S. 212; Heintz, *ibid.*, Bd. lxxxviii. S. 300.
[6] Bromeis, *ibid.*, 1842, Bd. xlii. S. 46.
[7] *Compt. rend. Acad. d. sc.*, Paris, tome civ.
[8] *Overs. o. d. k. Danske Vidensk. Selsk. Forh.*, Kjobenhavn, 1891.
[9] *Chem. News*, London, vol. lxiii.
[10] *Ztschr. f. Biol.*, München, Bd. xxxi. S. 1.
[11] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xix. S. 369.
[12] Duclaux (*loc. cit.*) found formic acid in cows' butter which had been exposed to sunlight.

acids contain equal quantities of caproic, caprylic, and capric acids, and the merest traces of butyric acid. The principal acids present, as is usual in animal fat, are palmitic, stearic, and oleic acids, and one or more acids of lower molecular weight, including myristic acid. The melting point of the mixture of acids is 37° to 39°, and of the fat itself 30° to 31° C.

The proteids of milk.—The proteids which occur in milk are three in number. The most abundant and most important of these is *caseinogen*. It is this proteid which is acted upon by rennet, and converted into *casein* or cheese.[1] The other two proteids are only present in small quantities; they are called lactoglobulin and lact-albumin. Proteoses and peptone were described in milk by many of the older workers. This was due to the use of faulty methods of analysis (see p. 41).[2]

Coagulation of milk.—When milk is allowed to stand at the ordinary temperature exposed to the air, the chief change it undergoes is the lactic acid fermentation. The acid formed precipitates a part of the caseinogen, but this is a different thing from the conversion of caseinogen into casein. Sometimes, however, certain aërobic bacterial growths act like rennet in causing a true curd. Certain of the higher plants (*Ficus*, etc.) also curdle milk.

The agency by which the clot is most readily formed is that of rennet. This is a ferment secreted by the stomach, and is usually obtained from the stomach of sucking animals, like the calf. The pancreatic juice also has a curdling action on milk (see p. 137), and extracts of many tissues (such as testis, liver, lung, muscle) have a feeble action of the same nature.[3]

Hammarsten[4] and, later, Friedberg[5] showed conclusively that the active principle of rennet is not pepsin; that it requires for its efficient action the presence of calcium salts, of which the phosphate is the one which is mostly present in the milk, and that it will act in a weakly acid, neutral, or alkaline solution. It acts most readily at 40° C., and is destroyed at 70° C. The ferment itself in the rennet extracts is termed chymosin by Friedberg, and rennin by Foster.[6]

When rennet is added to cows' milk the result is a coherent clot or curd, which expresses a clear yellowish fluid, the whey. The curd contains the fat entangled with the casein; the whey contains the other proteids, sugar, and salts of the milk. In human milk the curd is usually composed of smaller flocculi, and a similar flocculent coagulation can be produced in cows' milk by previously boiling it, or by diluting it. Lime water, soda water, or barley water are generally used as diluents for this purpose.

The coagulation of milk is somewhat analogous to that of blood, and the analogy is accentuated by the fact that in both cases calcium

[1] The utility of this nomenclature is at once apparent when casein and caseinogen are contrasted with fibrin and fibrinogen, myosin and myosinogen, even although the analogy is not complete in details. Hammarsten, however, prefers to call the proteid in milk, casein; while the coagulated proteid he terms, after Schulze and Röse (*Landw. Versuchs. Stat.*, Berlin, Bd. xxxi.), paracasein.

[2] For a critical article on the estimation of the various proteids in milk, see Schlossmann, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 197.

[3] Edmunds, *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. 465.

[4] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, S. 135.

[5] *Journ. Am. Chem. Soc.*, N. Y., 1888, p. 15.

[6] "Text-book," 5th edition, p. 519.

salts appear necessary, and that coagulation can be delayed or prevented by decalcifying the fluid. This is most readily done by adding a small quantity of a soluble oxalate.[1] Peptone has, as with blood, a retarding effect on coagulation.[2]

Green[3] has suggested that there is a definite relationship between the ferment and the calcium salt, resembling that which exists between pepsin and hydrochloric acid. Hammarsten[4] and, later, Ringer[5] showed what this relationship is. The formation of casein from caseinogen is, in fact, a double process: the first action is that of the ferment which converts the caseinogen into what we may call "soluble casein"; the second action is that of the calcium salt which precipitates the casein as curd, which is probably caseate of lime. This may be shown by taking a solution of caseinogen and adding rennet; if the mixture is warmed to 40° C., no visible change occurs; but nevertheless soluble casein, and not caseinogen, is now present. If the mixture is now boiled to destroy the ferment, cooled, and a drop of 2 per cent. calcium chloride added, the formation of a curd takes place.[6]

Casein and caseinogen differ in several of their properties. The curd of caseinogen precipitated by acetic acid is not nearly so coherent as the curd of casein produced by rennet. The precipitability of caseinogen by acid is not prevented by the addition of an oxalate, and there is 13 per cent. more calcium phosphate used up in rennet coagulation than in acid precipitation.[7]

The action of rennin upon caseinogen is not a simple conversion of that proteid into one of a more insoluble kind; but just as the fibrin ferment splits the molecule of fibrinogen into an insoluble proteid, fibrin, and a soluble globulin which passes into the serum, so rennin splits the caseinogen molecule into two parts: one part is the curd or casein; the other is a soluble proteid which passes into the whey, and is termed "whey proteid" by Hammarsten. This is the equivalent of the lacto-protein of other investigators. Some of these state it is like a proteose or peptone. It is certainly not coagulated by heat; it is precipitable by saturating with magnesium sulphate; rennet has no further action on it. It does not, however, give the pink biuret reaction.[8] It contains C., 50·3; and N., 13·2 per cent.[9]

Caseinogen.—This proteid may be precipitated from milk by the addition of acids like acetic, or by saturation with salts like sodium chloride and magnesium sulphate, or by half-saturation with ammonium sulphate. In all cases the fat of the milk is entangled with the precipitate. Caseinogen may be most readily prepared free from fat by first half-saturating the milk with ammonium sulphate; the precipitate is collected, well washed with half-saturated solution of the same

[1] Arthur and Pagès, *Arch. de physiol. norm. et path.*, Paris, Sér. 5, tome ii.; *Compt. rend. Soc. de biol.*, Paris, tome xliii. The addition of oxalates does not absolutely decalcify blood or milk; the calcium in close combination with the proteid remains unprecipitated. See Schäfer (*Proc. Physiol. Soc.*, 1895, p. xviii); Hammarsten (*Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 333), and also the article in this book on Blood.

[2] Edmunds, *loc. cit.*

[3] *Journ. Physiol.*, Cambridge and London, vol. viii. p. 371.

[4] "Zur Kenntniss des Kaseins," *Nova Acta Reg. Soc. Scient.*, Upsala, 1877. Festschrift.

[5] *Journ. Physiol.*, Cambridge and London, vol. xi. p. 464.

[6] Here the analogy of casein and fibrin breaks down. In blood coagulation the calcium salts assist in the genesis of the fibrin ferment rather than in the formation of fibrin from fibrinogen (Hammarsten, *loc. cit.*)

[7] D. F. Harris, *Journ. Anat. and Physiol.*, London, 1894, vol. xxix. p. 188.

[8] Halliburton, *Journ. Physiol.*, Cambridge and London, vol. xi. p. 462.

[9] Koster, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Bd. xi. S. 14.

salt, and then distilled water is added. This, in virtue of the salt adhering to the precipitate, dissolves out the caseinogen, and carries it through the filter, the greater part of the fat being left behind. From this solution the caseinogen is precipitated by acetic acid; it is collected, thoroughly washed, and dissolved in dilute alkali like lime water, and purified by repeated precipitation with acid and re-solution in alkali.

Ringer's method of obtaining caseinogen is a slight modification of that of Hammarsten: he precipitates caseinogen with acetic acid, collects and washes the precipitate, and grinds it up in a mortar with calcium carbonate; the mixture is thrown into excess of distilled water; the fat rises to the top; the chalk falls to the bottom, and the intermediate opalescent fluid is a solution of caseinogen. The separation into the three layers may be hastened by the use of the centrifuge.

In both cases, the caseinogen, if it has been thoroughly washed from soluble calcium salts, will not clot with rennet; the lime water in the one case and the calcium carbonate in the other not being sufficient to cause the separation of the curd: this, however, occurs immediately on the addition of a soluble salt of lime like the phosphate or chloride.

Solutions of caseinogen are not coagulated by heat. By prolonged heating they become opalescent; this often disappears on cooling. In some cases a scum forms on the surface, as in milk.

Caseinogen is not a globulin; still less is it an alkali albumin: it is a nucleo-albumin.

Analyses by Chittenden [1] gave the following result—C, 53·3; H, 7·07; N, 15·91; S, 0·82; O, 22·04. The amount of phosphorus was not estimated. Danilewsky [2] considered it to be a mixture of two proteids, but this, as Hammarsten [3] showed, was due to faulty methods of preparation. Chittenden made a study of the caseoses and proteoses obtainable from it by digestion.[4] Sebelien,[5] who also prepared casein peptone, states it is optically inactive—a most exceptional occurrence among proteids. The most interesting fact about its digestion by gastric juice, however, is, that it yields a precipitate of nuclein, or rather of pseudo-nuclein [6] (see pp. 65, 66).

The amount of and varieties of calcium phosphate in union with caseinogen and casein has been investigated by Soxhlet and Söldner [7] and by Courant.[8] Söldner describes two calcium compounds of caseinogen, containing respectively 1·55 and 2·36 per cent. of CaO; these are called dicalcium casein and tricalcium casein. Moraczewski [9] finds that the yield of pseudo-nuclein varies from 1·3 to 21·1 per cent. of the caseinogen employed; he finds that the amount of phosphorus in the pseudo-nuclein varies from 0·88 to 6·86 per cent. The whole phosphorus of the casein is not in the nuclein; the quantity in the nuclein is given as from 6 to 60 per cent. of the whole. This has been confirmed by Salkowski and Hahn.[10] These observers also find that

[1] *Stud. Lab. Physiol. Chem.*, New Haven, vol. ii. p. 156; iii. p. 66.
[2] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vii. S. 433.
[3] *Ibid.*, Bd. vii. S. 227.
[4] On peptonised milk see also Horton-Smith, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 42.
[5] *Centralbl. f. agric. Chem.*, Leipzig, 1889, S. 717.
[6] Moraczewski, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xx.
[7] *Loc. cit.* [8] *Loc. cit.*
[9] *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xx. S. 28.
[10] *Arch. f. d. ges. Physiol.*, Bonn, Bd. lix.

the pseudo-nuclein is partly soluble in gastric juice;[1] it is by far the most soluble of the nucleins,[2] though the majority are partly soluble after pancreatic digestion.[3]

Casein.—This name should be restricted to the proteid formed by the action of rennin, or of ferments that act like rennin. As a general rule, it is more insoluble than caseinogen; it is, however, readily soluble in dilute alkalis such as lime water. From these solutions it is readily precipitable by traces of calcium chloride; and also by sodium chloride (Hammarsten). The precipitate with calcium chloride increases on heating, but, like many calcium compounds, partially redissolves on cooling (Ringer).

The main distinction between casein and caseinogen is, however, that which was first insisted on by Hammarsten, namely, that caseinogen can be curdled by rennet, casein cannot. Some recent work by D. F. Harris [4] and Peters [5] appeared to cast doubt upon this essential distinction, and to suggest the possibility of recoagulation of casein, analogous to that of myosin. The fallacies into which these observers were drawn have been pointed out independently by Edmunds,[6] Hammarsten,[7] and R. Benjamin.[8] Peters, for instance, used a preparation of rennet, rich in sodium chloride and calcium salts; the precipitate he obtained by adding this to a solution of casein was due to these salts, not to the ferment.

Pancreatic casein.—An interesting variety of casein is that formed by the action of pancreatic juice on milk, which has been recently investigated by Brodie and myself.[9]

Kühne [10] was the first to point out that extracts made from the pancreas of the dog cause milk to coagulate; this action was described in some detail by Sir William Roberts.[11] Various conditions which influence the clotting were observed by Edkins,[12] and the occurrence of the action in pancreatic extracts from a number of animals determined by Harris and Gow.[13]

Our attention was drawn to the subject by a sentence in Prof. Gamgee's "Physiological Chemistry," [14] in which he points out that it does not necessarily follow that because extracts of the organ have a clotting action, the pancreatic juice possesses it also.

We accordingly performed experiments with the actual pancreatic secretion, obtained from temporary fistulæ in dogs, and our conclusions are summarised as follows:—

1. The pancreatic juice obtained from temporary pancreatic fistulæ, from dogs, produces a change in the caseinogen of milk.

[1] The nutritive value of casein is given by Marcuse (*Arch. f. d. ges. Physiol.*, Bonn, Bd. lxii. S. 223) as equal to that of meat proteids.

[2] E. Salkowski (*Virchow's Archiv*, Bd. cxliv.) states that caseinogen, if not coagulated in the process of preparation, is completely digested by gastric juice, if a sufficient volume of the latter is employed, *e.g.* 500 parts of gastric juice to 1 of caseinogen.

[3] Sebelien, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xx.; Popoff, *ibid.*, Bd. xviii.; Gumlich, *ibid.*, Bd. xviii.; Weintrand, *Verhandl. d. physiol. Gesellsch., Arch. f. physiol.*, Berlin, 1895; Clara Willdenow, Inaug. Diss., Bern, 1893; W. Sandmeyer, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xxi. S. 87.

[4] *Loc. cit.*

[5] Preisschrift, Rostock, 1894.

[6] *Loc. cit.*

[7] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 103.

[8] *Virchow's Archiv*, 1896, Bd. cxlv. S. 30.

[9] *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. S. 97.

[10] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, N. F., Bd. iii. S. 3.

[11] *Proc. Roy. Soc. London*, 1879 and 1881.

[12] *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 193.

[13] *Ibid.*, 1892, vol. xiii. p. 469.

[14] Vol. ii. p. 446.

2. This action differs from the action of rennet in the following particulars:—

(*a*) The precipitate of casein occurs in the warm bath (35°–40° C.) in the form of a finely granular precipitate, the milk to the naked eye undergoing no change in its fluidity. On cooling this to the temperature of the air, it sets into a coherent curd which contracts to only a small extent, and is again broken up into fine granules by warming to 35° C., the milk to the naked eye becoming again fluid. This may be repeated a great number of times.

(*b*) This phenomenon is not prevented, but only slightly hindered, by such an addition of potassium oxalate as completely inhibits the activity of rennet.

3. The experiments performed with extracts of the gland lead to similar results, which may be masked if the action of the tryptic ferment is very energetic.

4. The precipitate produced may be provisionally termed *pancreatic casein.* By the action of rennet it can be converted into true casein. Its solubilities, as summarised in the following table, are partly like those of caseinogen, partly like those of casein. It is probably something intermediate between the two.

	(*a*) Caseinogen.	(*b*) Casein.	(*c*) Pancreatic Casein.
(*a*) In water + $CaCO_3$	Soluble.	Insoluble.	Insoluble.
(*b*) In lime water .	Soluble; precipitable with difficulty by $CaCl_2$.	Soluble; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40°, soluble on cooling.	Soluble; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40°, not soluble on cooling.
(*c*) The precipitate produced by adding $CaCl_2$ to (*b*)	Soluble in 5 per cent. NaCl.	Insoluble in 5 per cent. NaCl.	Slightly soluble in 5 per cent. NaCl.
(*d*) Lime water solution.	Converted into casein by trace of phosphoric acid and rennet.	So readily precipitable by trace of calcium salts that the action of rennet could not be properly tested.	Behaves like caseinogen.
(*e*) In 0·5 sodium bicarbonate solution.	Soluble; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40°, dissolves on cooling.	Soluble; precipitable with difficulty by $CaCl_2$.	Soluble; precipitable with ease by $CaCl_2$; precipitate produced by trace of $CaCl_2$ at 40° C., not soluble on cooling.

The casein and caseinogen of human milk.—The facts described up to the present point are derived from experiments on cows' milk. There are very important differences between this and the principal proteid of human milk. A large number of investigators[1] have noted such differences as a more finely subdivided and more easily digestible clot formed by rennet, but the difference between the two proteids goes deeper than that. Human caseinogen is more difficult to precipitate by acids (and is easily soluble in excess) and by salts; it often will not

[1] Bredert and Schröter, *Centralbl. f. agric. Chem.*, Leipzig, 1888; Biedert, "Untersuch. ü. d. chem. Unterschiede der Menschen und Kuhmilch," Stuttgart, 1884; Langgaard, *Virchow's Archiv*, Bd. lxv.; Makris, Inaug. Diss., Strassburg, 1876.

clot with rennet at all; when it does so, the clot is a flocculent precipitate, which frequently redissolves rapidly in excess of gastric juice. According to Szontagh,[1] human caseinogen yields no pseudo-nuclein on gastric digestion; this was confirmed by Wroblewski,[2] who found also that human caseinogen has the following percentage composition—C, 52·24; H, 7·31; N, 14·9; P, 0·68; S, 1·117; O, 23·66. This, it will be seen, is different from the composition of the caseinogen of cows' milk. Human caseinogen contains phosphorus, but not in the form of pseudo-nuclein, as in cows' milk.

Wroblewski finds that human milk contains small quantities of lact-albumin, and of another proteid very rich in sulphur (4·7 per cent.) and poor in carbon (45·01 per cent.). Lehmann and Hempel[3] find that the caseinogen of cows' milk contains 7·2 per cent. of ash; this consists of CaO, 49·5; MgO, 2·4; P_2O_5, 47·0; and SO_3 1·06 per cent. The elementary composition of the proteid is given as C, 50·86; H, 6·72; N, 14·63; P, 0·81; S, 0·72; ash, 6·47 per cent. The caseinogen of woman's milk contains more sulphur, 1·09, and less ash, 3·2 per cent. Some of the differences between the two caseinogens are doubtless dependent on the amount and nature of the ash with which they are associated.

The occurrence of *nucleon* (phospho-carnic acid) in milk has already been mentioned on p. 104. Siegfried[4] states that the nucleon accounts for 41·5 per cent. of the phosphorus in human milk, but for only 6 per cent. of that in cows' milk. Practically all the phosphorus in human milk is in organic combination (nucleon and caseinogen).

Lact-albumin.—After the precipitation of caseinogen and lacto-globulin by half-saturation with ammonium sulphate, lact-albumium remains in solution. It can be incompletely precipitated from this solution by saturation with sodium sulphate. It is completely precipitated with the other proteids when milk is saturated with ammonium sulphate. It coagulates between 70° and 80° C.; in cows' milk at 77° C. It is not separable, like serum albumin and egg albumin, into several proteids by fractional heat coagulation. It, moreover, is coagulated by heat very slowly; the solution must be kept some hours at 77° C., before it is completely precipitated. Its specific rotatory power[5] $\alpha_D = -36°$, *i.e.* less than that of serum albumin; it has the following percentage composition: C, 52·19; H, 7·18; N, 15·77; S, 1·73; O, 23·13. The high percentage of sulphur is another distinction between it and serum albumin.

Lactoglobulin.—A trace of globulin is obtained from cows' milk by saturating it with magnesium sulphate, after the removal of the caseinogen by saturation with sodium chloride (Sebelien). Its characters are like those of serum globulin. The amount of globulin in colostrum is considerable, but in fully-formed milk it is present in so small an amount that for a long time I was unable to confirm Sebelien's statement. Hewlett,[6] however, who worked with me, confirmed its presence.

[1] *Ungar. Arch. f. Med.*, Wiesbaden, Bd. i. S. 192; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xxii. S. 168.

[2] Inaug. Diss., Bern, 1894. See also Moraczewski, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xx. S. 28.

[3] *Arch. f. d. ges. Physiol.*, Bonn, Bd. lvi. S. 558.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. xxii. S. 575.

[5] Sebelien, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xv. S. 184.

[6] *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 798. See also Arthus, *Arch. de physiol. norm. et path.*, Paris, 1893, p. 673.

Kemmerich[1] stated that casein (*i.e.* caseinogen) is formed at the cost of the albumin of milk after secretion. He estimated the caseinogen by precipitating it with dilute acetic acid, the precipitate being subsequently freed from fat by ether, dried and weighed. He estimated the albumin by weighing the heat coagulum after separating out the acetic acid precipitate, and he found that, after the milk is allowed to stand some hours at the body temperature, the caseinogen increases in quantity, and the albumin diminishes. Dähnhardt[2] claimed to have separated out from the cells of the mammary gland a ferment soluble in glycerine which hastens this process.[3]

These experiments are quoted with approval by Heidenhain,[4] but do not seem to have been followed up recently by the more precise methods of modern milk analysis. The differences noted by Kemmerich are usually small, and might be well within the limits of experimental error.[5] They date from a time when lact-albumin was considered to be identical with serum-albumin, and when caseinogen was looked upon as nothing more than alkali-albumin. Among other statements made by Kemmerich is the one that lact-albumin is converted into casein by boiling—an assertion which is quite sufficient to show the somewhat crude notions prevalent at the time concerning the proteids of milk. The dominant idea of these workers appears to be to account for the milk-proteids as simple derivatives of the blood proteids.

In the foregoing account of milk, no description of analytical processes has been given. For the numerous methods which may be used in this highly technical branch of analytical chemistry, the reader is referred to text-books on that science.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 401.
[2] *Ibid.*, 1870, Bd. iii. S. 586.
[3] J. C. Lehmann considered that caseinogen is formed from albumin by weak alkali (*Centralbl. f. d. med. Wissensch.*, Berlin, 1864, S. 530).
[4] Hermann's "Handbuch," 1883, Bd. v. S. 395.
[5] That this explanation is probably correct, is shown by some experiments of Schmidt-Mülheim (*Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxviii. S. 243) and Thierfelder (*ibid.*, 1883, Bd. xxxii. S. 619), who, by using the same methods, found a slight diminution of the casein after milk had stood some hours at the body temperature. Schmidt-Mülheim supposed that on standing some of the casein is converted into peptone.

THE BLOOD.

By E. A. Schäfer.

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The blood is a red fluid of alkaline reaction; in man its specific gravity is about 1·060. It has an odour which is different in different species of animals, and is brought out by the addition of sulphuric acid. It sets more or less rapidly into a solid clot or coagulum after death, or on removal from the living blood vessels. It consists of a clear, yellowish liquid, the plasma or liquor sanguinis, and of microscopic particles or corpuscles of two kinds: the one kind, less numerous, termed the white, or colourless, or lymph corpuscles (leucocytes); the other kind, by far the most numerous, the red, or coloured corpuscles (erythrocytes), which give the blood its characteristic tint. In addition to these, a variable number of much finer discoid colourless particles (elementary particles, blood-platelets) are apparent in a microscopic preparation of drawn blood.

Amount.—The amount of blood in the body was determined in the following manner by Welcker:[1]—A measured sample of blood is drawn, and, after being defibrinated, portions of it are diluted to different degrees to serve as samples of comparison. The rest of the blood is then collected and defibrinated, and the vessels are washed out with salt solution until the washings are colourless: they are all added to the defibrinated blood, which is now diluted with water until it corresponds in tint with one of the above samples, the dilution of which is accurately known. The total quantity of blood in the vessels can then be calculated. In order to obtain every trace of blood, Welcker further minced up the whole animal and extracted the tissues with water, adding this to the mass of blood. Some hæmoglobin would thereby, however, be yielded by the muscles (Kühne).

The amount has also been determined during life by the method of Gréhant and Quinquaud,[2] who allowed an animal to inspire a

[1] *Ztschr. f. rat. Med.*, 1858, Ser. 3, Bd. iv. S. 147. Welcker's method is improved by combining the hæmoglobin with carbonic oxide gas (Gescheidlen).

[2] *Compt. rend. Acad. d. sc.*, Paris, 1882, tome xciv. p. 1450; *Journ. de l'anat. et physiol. etc.*, Paris, 1882, No. 6, p. 564.

measured amount of carbonic oxide (mixed with oxygen); then drew off a measured quantity of blood, and determined the amount of carbonic oxide this contained; the amount in the whole of the blood in the body would be in the same proportion, and the quantity of blood could thus be calculated. The result arrived at by these two methods is that the blood is equal to one-eleventh to one-fourteenth of the body weight (about $5\frac{1}{2}$ kilos. in a man of 70 kilos.).

Colour: laking of blood.—The colour of the blood varies in different parts of the vascular system. The differences are dependent upon the amount of oxygen in combination with the hæmoglobin. The colour also becomes altered by any reagent or circumstance which tends to cause the hæmoglobin to pass out from the corpuscles into the circumjacent fluid. When this is brought about, the blood loses its opaque appearance and becomes transparent and of a laky tint. Such "laky" blood is readily produced by the addition of distilled water, and also by water holding neutral salts in solution up to a certain percentage; which percentage varies for different salts, and also, with the same salts, for the blood of different animals. A solution containing just such a percentage of salt as suffices to keep the corpuscles unaltered in form, and without removal of any of their hæmoglobin, is "isotonic";[1] solutions below and above such strength are respectively "hypisotonic" and "hyperisotonic."[2] For human blood, a solution of common salt is isotonic with a percentage of 0·9; for defibrinated ox blood, with 0·6, and about the same for frog's blood. Very slight differences of external condition will tend to alter the permeability of the blood corpuscles both for hæmoglobin and for other substances. A minute diminution in the alkalinity, such as is produced by the addition of 0·003 per cent. HCl, so alters the permeability as to cause proteid to pass from the corpuscles into the serum, and chlorides or phosphates to pass into the corpuscles from the serum; a minute increase of alkalinity has the opposite effect. The passing of oxygen and carbonic acid respectively through blood produces like physical changes, and it has been suggested that these changes may come into operation in connection with the metabolic exchanges in the capillaries.[3] These osmotic effects alter the total volume of the corpuscles as compared with the plasma; the proportional alterations are determined by centrifugalising blood, and then measuring the respective amounts of subsided corpuscles and superjacent plasma.[4] Laky blood is produced not only by water and dilute solutions of neutral salts, but also by many other reagents or conditions, such as crushing of the corpuscles, freezing and thawing the blood, and also by the action of acids, of alkalies, of bile salts, of ether and chloroform, of heat and electricity.[5] In all cases the permeability of the envelope of the red corpuscle (see p. 154) becomes altered either by mechanical means or by the solution of one or more of its constituents,

[1] Having the same osmotic pressure (de Vries, *Ztschr. f. physikal. Chem.*, Leipzig, 1888, Bd. ii. S. 415).

[2] Hamburger, *Arch. f. Physiol.*, Leipzig, 1886, S. 476; *Ztschr. f. Biol.*, München, 1890, Bd. xxvi. S. 414.

[3] Hamburger, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 405; *Arch. f. Physiol.*, Leipzig, 1892, S. 513; 1893, Suppl. Heft, S. 153; and *Verhandel. d. k. Akad. v. Wetensh. te Amsterdam*, 1897, S. 368.

[4] Koeppe, *Arch. f. Physiol.*, Leipzig, 1895, S. 154; Hedin, *Skandin. Arch. f. Physiol.*, Leipzig, 1895, Bd. v. S. 207 and 238.

[5] For literature of this, see Rollett in Hermann's "Handbuch der Physiologie," Bd. iv. S. 14.

and the hæmoglobin is thereby permitted to diffuse into the circumjacent fluid.

Specific gravity.—The specific gravity of the blood varies in health within small limits, namely, for men, 1057 to 1066; for women, 1054 to 1061.[1] According to Lloyd Jones,[2] it is lower than this in women, averaging 1051·5 between the ages of 35 and 45, whereas in men of the same age it averages 1058·5. It falls a little when much fluid is injected, and is raised a little by profuse perspiration, but the changes thus produced are very small.[3] It is slightly less in children than in adults, but it is higher in the fœtus than in the mother; and it is highest in the child at term, in which it is 1066, the specific gravity of the maternal blood being then only about 1050.[4] The diurnal variations are normally so small as to be almost negligeable. Passive congestion of the part from which the specimen examined is taken increases the specific gravity, whereas active congestion lowers it. It varies also according to the part of the body from which it is taken, such variation being probably due to accidental admixture with lymph. Thus Lloyd Jones found a difference of as much as three or four per 1000 between blood from the finger (lower) and blood from the skin over the shin (higher). Of the animals examined, it has been found higher in birds than mammals, and to vary somewhat in these animals in different species. The variations in age and sex are closely related to variations in the amount of hæmoglobin. Saline solution (NaCl, 0·75 per cent.) injected in quantity into the blood only depresses the specific gravity for a short time. The specific gravity of blood from a vein is practically the same as that from the corresponding artery, if care be taken to avoid venous congestion.[5]

The specific gravity of the blood falls after the removal of blood, doubtless from absorption of the specifically lighter lymph from the tissues. It subsequently (in about six hours) not only returns to normal, but even rises above normal; after about twelve hours it has permanently recovered its normal specific gravity.[6]

Almost any operation performed upon an animal, especially one involving exposure or irritation of a serous membrane, will produce an increased percentage of corpuscles (polycythæmia), or a corresponding diminution of plasma. This is due, not to increased formation of corpuscles, but to exudation of plasma in the inflamed or irritated part.[7]

[1] Hammerschlag, *Ztschr. f. klin. Med.*, Berlin, 1892, Bd. xx. S. 444. The results of other workers will be found in this paper. For the older literature, see Rollett, *op. cit.*, S. 134. The numbers given by Peiper (*Centralbl. f. klin. Med.*, Bonn, 1891, Bd. xii. S. 217) are 1·055 as the average for men, and 1·053 for women.

[2] *Journ. Physiol.*, Cambridge and London, 1888, vol. viii. p. 1.

[3] Schmaltz, *Arch. f. klin. Med.*, Berlin, 1891, Bd. xlvii. S. 145; Grawitz, *Ztschr. f. klin. Med.*, Berlin, Bd. xxi. S. 459, and Bd. xxii. S. 411.

[4] Lloyd Jones, *op. cit.*

[5] Cohnheim and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 303. For the effects of varying conditions of health and disease upon the specific gravity of the blood consult Lloyd Jones, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 299, where a large number of observations are accumulated. Lloyd Jones worked by Roy's method. Scholkoff (Diss., Bern, 1892), working by a different method (pycnometer), has obtained very similar results. Both observers agree in the important fact that the specific gravity varies as a rule *pari passu* with the richness in hæmoglobin. For the specific gravity in different animals, and for variations experimentally induced, see Sherrington and Copeman, *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 52.

[6] Ziegelroth, *Virchow's Archiv*, 1895, Bd. clxi. S. 395.

[7] See on this subject, Löwit, "Studien z. Phys. u. Path. d. Blutes," Jena, 1892; W. Hunter, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. S. 115; Sherrington and Copeman, *loc. cit.*; Sherrington, *Proc. Roy. Soc. London*, 1893, vol. lv. p. 161.

The methods which have been used for determining the specific gravity of the blood are—(1) that of directly weighing a sample (pycnometer), and (2) Roy's method. The latter is by far the readiest, and, for small quantities of blood, the more accurate. It consists in transferring minute drops of blood to glycerine and water, mixed in varying proportions, and forming a graduated series of liquids of different and known specific gravities, and in observing in which mixture the drop tends neither to rise nor to fall. The method has been modified by the use of benzene and chloroform mixtures instead of glycerine and water, and also by placing the drop of blood in such a mixture, and adding benzene or chloroform, as the case may be, until the drop remains exactly suspended, tending neither to rise nor fall; the specific gravity of the mixture is then taken (Hammerschlag). It may be doubted, however, whether these modifications are more readily applied, or more accurate than Roy's method.

Reaction.—The alkaline reaction of the blood is easily recognised, in spite of its red colour, by applying a drop of blood to the surface of a piece of glazed litmus paper, and after half a minute wiping away the blood with a piece of clean linen, wetted with distilled water or with neutral salt solution. The part of the paper which was covered by the blood will show a blue patch.[1] A comparison may be made between different samples of blood, by using a series of litmus papers which have been reddened by standard acid of different strengths.[2] For estimating the amount of its alkalinity the blood is mixed in small measured quantity with a solution of sulphate of soda, containing a definite amount of tartaric acid,[3] titrated against sodium hydroxide, and the mixture found which is exactly neutral to glazed litmus paper. Tested by this method,[4] the alkalinity of human blood is found to be equal to about 0·200 grms. of sodium hydroxide per 100 c.c. blood.[5] There appears to be a diurnal variation, the alkalinity being lowest in the morning, and gradually rising in the afternoon, becoming less again in the evening. It rises during digestion.[6] It is diminished by muscular work, especially with a diet containing little or no proteid.[7] On the other hand, with a diet rich in proteids, it undergoes very little alteration. In accordance with this, it is found that carnivora resist an artificial diminution of the normal blood alkalinity (such as would be caused by giving dilute mineral

[1] Schäfer, *Journ. Physiol.*, Cambridge and London, 1881, vol. iii. p. 292.

[2] Haycraft and Williamson, *Proc. Roy. Soc. Edin.*, 1888, vol. xv. p. 396. For fallacies in the clinical application of this method, see Hutchison, *Lancet*, London, 1896, vol. i. p. 616.

[3] Lassar, *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. ix. S. 44; Drouin (Thèse, Paris, 1892) used oxalic acid.

[4] The principle of the method is due to Zuntz, who, however, used phosphoric acid (*Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 801); but the details were greatly improved by Landois ("Real-Encyklopädie," Aufl. 2, Bd. iii., article "Blut"). For other methods of estimating the alkalinity, see v. Limbeck, *Wien. med. Bl.*, 1895, S. 295; and Schutz-Schultzerstein, *Centralbl. f. d. med. Wissensch.*, Berlin, 1894, Bd. xxxii. S. 801. According to Mayer (*Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1883, Bd. xvii. S. 304), all titration methods are unreliable with blood, but his conclusions have not been accepted by most physiologists.

[5] Freudberg, *Virchow's Archiv*, 1891, Bd. cxxv. S. 566, gives an average alkalinity in health of 0·200 to 0·240 grm. NaHO per cent. Jeffries (*Boston Med. and S. Journ.*, 1889) obtained about 0·200 as the average, and Drouin about 0·206. v. Jaksch. (*Ztschr. f. klin. Med.*, Berlin, 1888, Bd. xiii. S. 353) found the alkalinity of normal human blood as high as 0·260 to 0·300.; Loewy (*Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviiii. S. 498), working with "laked" blood, found its alkalinity = 0·449 grms. NaHO; and Berend (*Ztschr. f. Heilk.*, Berlin, 1896, S. 351) obtained an alkalinity from "laked" blood of 0·450 to 0·500.

[6] Peiper, *Virchow's Archiv*, 1889, Bd. cxvi. S. 337.

[7] Cohnstein, *Virchow's Archiv*, 1892, Bd. cxxx. S. 332. See also Geppert u. Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 233, and Peiper, *loc. cit.*

acids by the mouth), whereas herbivora show no such resistance.[1] The result appears to be due to the fact that ammonia becomes split off from the superabundant proteid in place of urea, and serves to unite with and neutralise the excess of acid.

Kraus found the alkalinity to be diminished by laking the blood, *i.e.* the blood to become more acid after the corpuscles are broken up, but Loewy and others have found it to be very high under these conditions; alkaline substances in the corpuscles coming also into estimation.[2] It is diminished after withdrawal, and during the process of coagulation (Zuntz);[3] when this is completed, it is about ·04 grm. NaHO less. The alkalinity is also diminished in fever and in many diseases. In diabetic coma[4] and in the cold stage of cholera[5] an acid reaction has even been detected. The diminution of alkalinity is accompanied by a diminished amount of carbonic acid in the blood.

The alkalinity is usually stated to be due to carbonate and phosphate of soda. This may be true for the alkalinity of the plasma, but it is insufficient to account for that of the corpuscles as well, and in them is probably largely due to the presence of organic substances of weak basic nature. Thus it was found by Zuntz and Lehmann,[6] that whereas a sample of calcined blood showed an alkalinity equivalent to 0·240, and the estimation of the alkalinity of the same blood by the amount of carbonic acid it would combine with gave an alkalinity equal to 0·276, the estimation by titration of the same blood after laking gave a result as high as 0·832. Saturation of blood with carbonic acid causes the corpuscles to become less and the serum more alkaline.[7]

Although the blood is alkaline in reaction to litmus, it contains salts (hydrodisodic phosphate and sodic bicarbonate) which are theoretically acid,[8] having the power both of fixing bases and of turning other acids out of combination (Rollett). In this sense the "acidity" as well as the "alkalinity" of the blood can be spoken of. According to Kraus[9] it is normally equivalent in venous blood to from 0·162 to 0·232 grm. NaHO per 100 grms. blood; being increased in conditions of fever to 0·272 grm., and in diabetic coma to 0·347 grm.

Coagulation.—The blood begins to coagulate three or four minutes after it is drawn, and the process is completed in seven or eight minutes.[10] The process is hastened by warmth, by agita-

[1] Walter, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1877, Bd. vii. S. 148.

[2] Kraus, *Ztschr. f. Heilk.*, 1890, Bd. x. S. 106; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxvi. S. 186; Winternitz, *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 505; Loewy, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. viii. S. 462; also Loewy and Zuntz, *ibid.*, S. 511, and Lehmann, *ibid.*, S. 428. See note on p. 144.

[3] See also Loewy and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 507.

[4] Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xix. S. 209; *Mitth. a. d. med. Klin. z. Königsberg*, Leipzig, 1888, S. 174.

[5] C. Schmidt, "Charakt. d. epid. Cholera," Leipzig, 1850; Straus, Roux, Thuiller et Nocard, *Compt. rend. Soc. d. biol.*, Paris, 1883, S. 569.

[6] *Arch. f. Physiol.*, Leipzig, 1893, S. 556.

[7] Zuntz in Hermann's "Handbuch," 1880, Bd. iv. Th. 2, S. 77.

[8] Maly, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1878, Bd. lxxvi. Abth. 2, S. 21; and *ibid.*, 1882, Bd. lxxxv. Abth. 3, S. 314.

[9] *Op. cit.*

[10] Hewson, "Properties of the Blood," 1772. In "Works," edited by G. Gulliver for the Sydenham Society, p. 24. Blood from the hepatic veins coagulates rather more slowly than blood from other parts of the vascular system. Paulesco (*Arch. d. physiol. norm. et path.*, Paris, 1897, p. 21) states that blood from the portal vein from animals in full digestion of proteid food may take as long as fifty minutes to coagulate, but otherwise there is little difference in blood from different vessels. For a method of accurately estimating the time of commencing coagulation, see Brodie, *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 403.

tion, by contact with foreign matter, by moderate dilution with water, by addition of calcium salts, and by fibrin ferment and nucleo-proteids. It is delayed by cold, by dilution with solutions of neutral salts or of sugar, by intravenous injection of albumose, and of various other organic substances, such as diastatic ferments; also by prevention of contact with foreign matter, as by drawing it into oil. It is also prevented if the soluble lime salts are precipitated by soluble oxalates, by fluorides, or by soap. A temperature of 56° C. prevents coagulation by precipitating the fibrinogen upon which the coagulation depends. It remains fluid for an indefinite time within the living blood vessels, even in a portion of vessel which has been isolated by ligatures. But if the inner surface of any blood vessel is injured, the blood tends to deposit a coagulum upon the injured part. And if a foreign substance is introduced into a blood vessel a clot forms upon it.

It also coagulates within the vessels of a living animal if a solution of nucleo-proteids is injected in a certain amount into the veins (Wooldridge);[1] but if the amount injected is too small to cause coagulation, the opposite effect is obtained, the coagulability being temporarily destroyed (negative phase, Wooldridge). These effects are not peculiar to nucleo-proteids, but have been shown to be also produced by intravenous injection of artificially prepared "colloids"[2] (see p. 37), and by snake-venom.[3]

If the coagulation is prevented by any of the above means, the corpuscles, which are heavier than the plasma, tend to fall to the bottom of the vessel, and to leave the upper layers of plasma clear. At the junction between the mass of subsided red corpuscles and the plasma is a "buffy" layer containing most of the white corpuscles. The subsidence may be accelerated by centrifugalising the blood. If cold be used to delay the coagulation, or if the blood be contained in a ligatured vein, carefully removed from an animal immediately after death, and suspended in a glass vessel, pure plasma may be drawn off from the upper layer completely free from red corpuscles, but usually containing a few leucocytes. The experiment is best performed with horse's blood, the corpuscles being relatively heavier in this as compared with that of other animals. This plasma clots on being placed in a glass vessel at the temperature of the air, but much more slowly than a sample of the original blood, and the more slowly the fewer the blood platelets and leucocytes it contains. If a sample be taken from the buffy layer—containing, therefore, many leucocytes and many blood platelets—the clotting is speedy and firm. If bird's blood is rapidly and repeatedly centrifugalised, plasma is obtainable almost entirely free from corpuscles, and no clotting occurs in it for days on standing in a glass vessel.[4] It appears, therefore, that the coagulation is independent of the red corpuscles, and is dependent upon the plasma and white corpuscles, and perhaps also upon the blood platelets. It is also dependent upon the presence of calcium salts. The exact relations which these factors bear to one another in the phenomenon of coagulation will be discussed later in considering the properties of fibrinogen.

The delay of coagulation produced by neutral salts is best obtained

[1] *Proc. Roy. Soc. London*, 1886, vol. xviii. p. 186; *Arch. f. Physiol.*, Leipzig, 1886, S. 397.

[2] Pickering, *Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. (*Proc. Physiol. Soc.*, p. v); and Halliburton and Pickering, *ibid.*, 1895, vol. xviii. p. 285.

[3] C. J. Martin, *Journ. and Proc. Roy. Soc. New South Wales*, Sydney, 1895.

[4] Delezenne, *Compt. rend. Soc. de. biol.*, Paris, 1896, p. 782.

by allowing the blood as it flows from a cut artery to mix with an equal volume of saturated solution of sulphate of soda or with a 10 per cent. solution of sodium chloride, or with one-third its bulk of a saturated solution of sulphate of magnesia. The plasma obtained after subsidence of the corpuscles is in these cases diluted with the salt solution (salted plasma), and may remain indefinitely uncoagulated. But, on diluting it with a sufficient amount of water, coagulation will usually occur. The delay produced by albumoses (commercial "peptone" is generally used) is obtained by injecting these, in the proportion of 0·3 grm. per kilog. of body weight into the circulating blood of a dog or cat.[1] The effect is not got in the rabbit.[2] Malt diastase and emulsin in somewhat larger quantity have a similar effect.[3] The blood of such a "peptonised" animal does not clot on being drawn, but it coagulates on passing carbonic anhydride through it, or on diluting it with water. Extract of leech-heads,[4] which contains an albumose,[5] and also extract of crayfish muscle (Heidenhain), act similarly in preventing coagulation, but in smaller doses. Leech extract does not, however, act exactly in the same manner as albumose, for the latter does not arrest coagulation if added in moderate quantity to drawn blood, whereas leech extract does arrest it (Haycraft).

To hinder coagulation by removal of lime salts, the blood is mixed as it flows from the vessels with a small amount of solution of sodium oxalate; 1 part of the salt to 1000 parts of blood is sufficient.[6] The corpuscles usually subside very readily in oxalated blood, and a clear plasma, nearly but not quite free from soluble lime salts, is easily got from it, coagulating quickly on the addition of chloride of calcium. It is not, however, the case that, as Arthus has asserted, oxalated blood or plasma always remains indefinitely uncoagulated without the addition of lime salts, for on allowing it to stand a few days a clot is frequently found in it.[7]

All the above methods yield plasma, either pure or in a somewhat modified condition. To obtain the blood corpuscles free from plasma it is necessary, after drawing off the superjacent fluid from them, to mix them with a further quantity of the salt solution used to prevent coagulation (*e.g.* 10 per cent. NaCl), and again to centrifugalise. Or the blood may be mixed as soon as drawn with a sufficient quantity of isotonic salt solution to delay its coagulation, and centrifugalised. By repeating the process several times the corpuscles may be got free from plasma, and may thus be analysed separately from the liquor sanguinis. But it is by no means certain that they have not undergone some alteration in composition by diffusion. Hitherto no means has been devised for meeting this objection.

Relative amount of plasma and corpuscles.—The relative amounts of plasma or serum and corpuscles can therefore only be found approximately by weighing the corpuscles obtained by this method from a given amount of blood. Indirectly, it has been arrived at for defibrinated blood by Hoppe-Seyler, by determining the percentage amount of

[1] Schmidt-Mülheim, *Arch. f. Physiol.*, Leipzig, 1880, S. 33.

[2] Fano, *ibid.*, 1881, S. 276.

[3] Salvioli, *Arch. per le sc. med.*, Torino, 1888, vol. xii. p. 245.

[4] Haycraft, *Proc. Roy. Soc. London*, 1884, vol. xxxvi. p. 478. Haycraft showed that leech extract acts by destroying fibrin ferment.

[5] Dickinson, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 566.

[6] Arthus et Pagès, *Arch. de physiol. norm. et path.*, Paris, 1890, p. 739.

[7] This is certainly so with the plasma obtained from oxalated dog's blood and sheep's blood (Schäfer, *Proc. Physiol. Soc.*, *Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. p. xx).

proteids in the serum, and of proteids and hæmoglobin in the subsided corpuscles, and in the whole blood respectively; and, calculating from the results obtained, the amount of plasma and of corpuscles respectively in 100 grms.[1] An earlier method consisted in determining the amount of fibrin in a given quantity of the whole blood, and of the plasma respectively, and from this calculating the percentage amount of plasma in the sample of blood.[2] Bunge determined the proportions in a similar manner by estimating the sodium in a sample of blood, and also in plasma of the same blood. This method is only applicable to certain animals (horse, pig) which have no sodium in their blood corpuscles.

The following example of the application of these methods is given by Bunge:[3]—

(*A*) By Hoppe-Seyler's method:—

In 100 grms. of defibrinated pig's blood were found—

(*a*) 18·92
(*b*) 18·88 } mean: 18·90 grms. proteids + hæmoglobin.

In the blood corpuscles of 100 grms. of the same blood—

(*a*) 15·04
(*b*) 15·13 } mean: 15·07 grms. proteids + hæmoglobin.
(*c*) 15·05

In the serum of 100 grms. of blood were—

18·90 − 15·07 = 3·83 grms. proteids.

In 100 grms. of serum—

(*a*) 6·74
(*b*) 6·79 } mean: 6·77 proteids.

From this the amount of serum in 100 grms. of the defibrinated blood may be computed—

6·77 : 3·83 : : 100 : 56·5.

Therefore 100 grms. blood contained 56·5 parts serum and 43·5 corpuscles.

(*B*) By estimation of sodium—

In 100 grms. of the whole blood of the same pig was found—

(*a*) 0·2403
(*b*) 0·2409 } mean: 0·2406 grms. Na_2O.

In 100 grms. of serum—

(*a*) 0·4283
(*b*) 0·4260 } mean: 0·4272 grms. Na_2O.

0·4272 : 0·2406 : : 100 : 56·3.

Therefore, by this method, 100 grms. blood contained 56·3 parts serum and 43·7 corpuscles—a result which agrees closely with that obtained by Hoppe-Seyler's method.

Similarly, in horse's blood, Bunge found by Hoppe-Seyler's method 46·5 per cent. serum, and 53·5 corpuscles, and by the sodium method 46·9 serum and 53·1 corpuscles.

A rapid approximate determination may be made by Blix's method (hæmatocrit).[4] The blood is mixed with a definite amount of 2½ per cent. potassium bichromate, and centrifugalised. The corpuscles rapidly accumulate at the bottom in an almost solid mass, and their

[1] "Handb. d. physiol. chem. Analyse," Aufl. 2, Berlin, 1865.
[2] Hoppe, *Virchow's Archiv*, 1857, Bd. xii. S. 483.
[3] "Physiol. and Pathol. Chemistry," trans. by Wooldridge, 1890, pp. 243, 244.
[4] Hedin, *Skandin. Arch. f. Physiol.*, Leipzig, 1890, Bd. ii. S. 134. Gaertner, *Berl. med. Wchnschr.*, 1892, No. 36, p. 890.

collective volume can be directly read off.[1] The estimation can be made with a small quantity of blood, and is therefore capable of being used for clinical purposes. The average percentage of corpuscles in human blood, as obtained by these several methods, is about 48, or very nearly one-half of the entire amount of blood. In the horse it is 53 per cent., in the pig 43·5 per cent., in the dog 35·7 per cent., and in the ox 32 per cent. Hedin obtained in himself an average percentage total corpuscular volume of 51, the greatest differences in his own blood being 54·4 and 48 per cent.; but the average for a large number of adult males was 48 and of females 43·3. In children of 6 to 13 years the amount was 45 per cent.

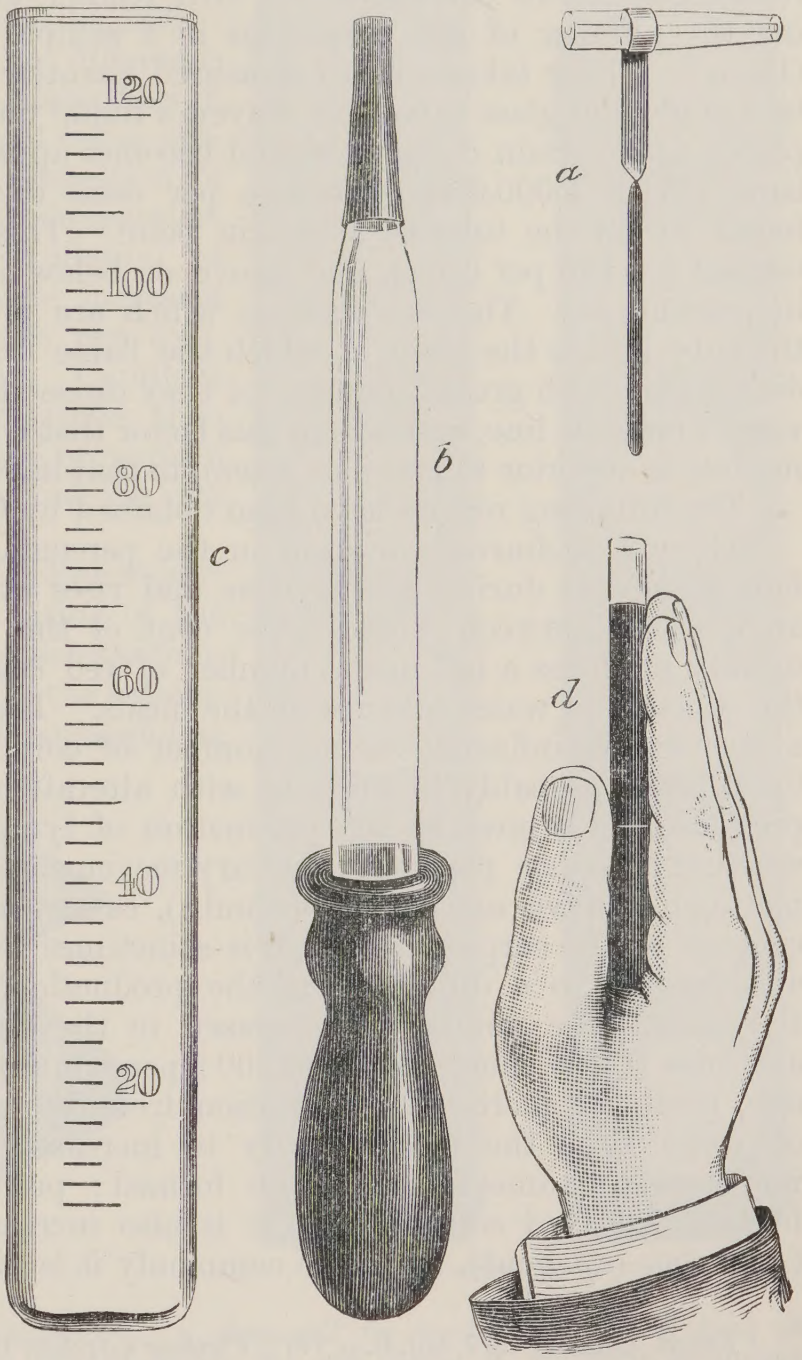

FIG. 21.—Oliver's apparatus for estimating the number of blood corpuscles. *a*, measuring pipette; *b*, dropper to contain Hayem's fluid; *c*, mixing tube graduated in percentages; *d*, mode of making the observation. (This must be done in a dark room.) *a*, *b*, and *c* are natural size.

Number of corpuscles.—The number of red corpuscles in a cubic millimetre of blood was determined by Vierordt and Welcker to be about 5,000,000 in adult men. There are rather fewer in women (about 4,500,000). Vierordt's method consisted in diluting the blood with a known amount of fluid which would preserve the corpuscles, and counting the number in a measured amount of the mixture. The same method is still in use, but its application has been greatly simplified in the

[1] An indirect method, based on the principle of centrifugalising blood with varying amounts of salt solution, and determining the organic nitrogen in the supernatant fluid, has been introduced by M. and L. Bleibtreu (*Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. li. S. 151), who claim to be able to estimate by its aid, not only the total corpuscular volume, but even the average volume and weight of a single blood corpuscle. The method has, however, been sharply criticised. (Hamburger, *Centralbl. f. Physiol.*, Leipzig, 1893, Bd. vii. S. 161; and *Virchow's Archiv*, 1895, Bd. cxli. S. 230; Eyckmann, *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lx. S. 340; and Hedin, *ibid.*, S. 360). See further, on the same subject, Lange, *ibid.*, 1892, Bd. lii. S. 427, and Bleibtreu, *ibid.*, Bd. lx. S. 405.

blood-counting apparatus (hæmacytometer) of Gowers and of Thoma.[1] The hæmatocrit can also be employed, since it has been determined (by exact enumeration) that each volume per cent. shown by that instrument represents 97,000 corpuscles. A still readier mode of rapidly estimating the number of red corpuscles in a sample of blood is that of G. Oliver.[2] Oliver takes a small measured quantity of blood and mixes it in a graduated glass tube with Hayem's fluid,[3] until the flame of a candle placed at a certain distance behind becomes apparent through the mixture. With 5,000,000 corpuscles per cent. the mixture will now be found to fill the tube to a certain point. This point is taken as the normal (or 100 per cent.), and above or below it the tube is graduated in percentages. The fine flutings which are produced in drawing out the tube enable the point at which the flame first becomes visible to be determined with great accuracy, for they cause it to appear as a transverse luminous line, and it is in this factor that Oliver's application of the method is superior to previous attempts that have been made to apply it.

The following results have been obtained by Oliver and others:—

There is a diurnal variation in the percentage of corpuscles, which falls somewhat during the daytime and rises at night. This variation amounts to between 4 and 5 per cent. of the normal number. Food usually produces a fall in the number of red corpuscles, independent of the amount of water taken with the meals. The posture of a limb has a considerable influence on the number of corpuscles obtained from it by a prick, probably in keeping with alterations in the intracapillary pressure, which governs the production of lymph. Muscular exercise, whether active or passive (voluntary movements, electrical stimulation, massage, passive movement of limbs), causes an increase in the percentage of the corpuscles, which is sometimes very marked.[4] This may also be due to a difference in the production and flow of lymph in the part. The number is increased in the case of residents in high altitudes (to as much as 8,000,000! per c.mm.).[5] This appears to be due, partly to increased evaporation from the general surface, and loss of water from the blood; partly to increased arterial tension, which increases the amount of lymph formed; probably not to increased formation of red corpuscles.[6] It is also increased in certain diseased conditions (*e.g.* gout), but more commonly it is diminished in disease.

[1] *Lancet*, London, 1877, vol. ii. p. 797; *Virchow's Archiv*, 1882, Bd. lxxxvii. S. 201. The older literature is given by Rollett in Hermann's "Handbuch," 1880, Bd. iv. Th. 1, S. 27–31.

[2] G. Oliver, Croonian Lectures, *Lancet*, London, 1896, vol. i.

[3] Distilled water, 200 c.c.; sulphate soda, 5 grms.; common salt, 1 grm.; corrosive sublimate, 0·5 grm. See Hayem ("Du Sang," Paris, 1889), where will be found an extended series of observations upon the microscopical characters of the blood.

[4] Noted also by Malassez, *Compt. rend. Soc. d. biol.*, Paris, séance du 31 Oct. 1874, *Gaz. méd. de Paris*, 1874, p. 573. For numerous other observations by this author consult "De la numération des globules rouges du sang," Paris, 1873, and papers in *Arch. de physiol. norm. et path.*, Paris, 1874, *et seq.*

[5] Viault, *Compt. rend. Acad. d. sc.*, Paris, 1890, tome cxi. p. 917; and 1891, tome cxii. p. 295. Oliver (Croonian Lectures, *Lancet*, London, 1896, vol. i. p. 1782) gives a useful epitome of what is known at present on this subject, together with many original observations.

[6] Grawitz, *Berl. klin. Wchnschr.*, 1895, S. 713 and 740. Cf. also A. Fick, *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lx. S. 589. Grawitz points out that at altitudes below 16,000 feet there is no need for a compensatory increase in number of red corpuscles, since the experiments of Fränkel and Geppert have shown that in dogs subjected to an atmospheric pressure, equal to that at this altitude, there is just as much oxygen taken up by the blood as at the ordinary pressure. Müntz found relatively more iron in the blood of rabbits and sheep from near the top of the Pic du Midi than in others living in the

Stierlin[1] found individual variations in healthy men, amounting to 1,650,000, and in healthy women to 2,230,000 per c.mm. E. Schiff[2] obtained more than 5,500,000 per c.mm. in new-born children: as development progresses, the number gradually sinks to about 5,000,000.

There is normally no difference between the number of corpuscles in corresponding arteries and veins, provided there exists no congestion of the part due to venous obstruction. In such a case the exudation of lymph from the capillaries increases the number of corpuscles per cent. in the blood of the vein. Capillary blood is poorer in corpuscles than that of the trunks, but the proportion varies with their width and the rate of the blood stream.[3]

Equally important for clinical purposes, with the determination of the number of red blood corpuscles as compared with the normal, is the estimation of the amount of hæmoglobin,

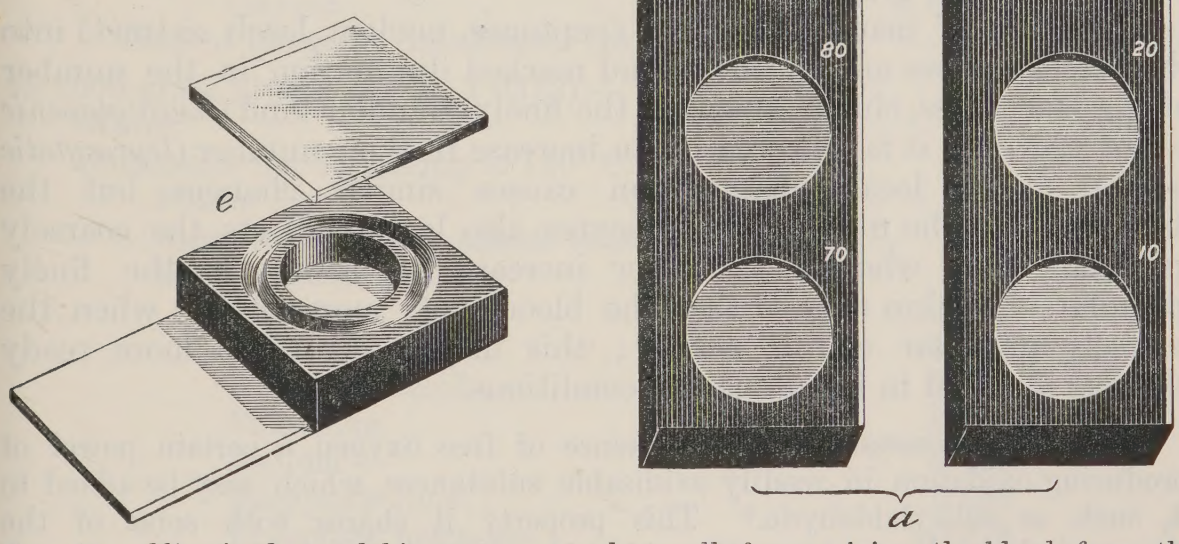

FIG. 22.—Oliver's hæmoglobinometer. *e*, glass cell for receiving the blood from the pipette: the dilution is effected within the cell itself. *a*, standard graduations made of tinted glass. To avoid multiplying these unduly they are furnished in tens per cent., the intermediate divisions of the scale being obtained by superposing tinted glass riders in a graduated series from 1 to 9. (These riders are not represented in the figure). The apparatus is shown of the natural size.

and the consequent determination of the proportionate amount of hæmoglobin per blood corpuscle. This may be expressed as a quotient thus:—

$$\frac{\text{percentage amount of hæmoglobin}}{\text{percentage number of corpuscles}} = \frac{100}{100} = 1 \text{ or normal}$$

at least theoretically: practically it is found to vary in health from

plains (*Compt. rend. Acad. d. sc.*, Paris, 1891, tome cxii. p. 298). Weiss, who kept rabbits at high altitudes for about four weeks, and compared them with control animals at lower levels, found an increase of corpuscles to the extent of 12 to 24 per cent., but no absolute increase of hæmoglobin in the whole body (*Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. xxii. S. 526).

[1] *Deutsches Arch. f. klin. Med.*, Leipzig, 1889, Bd. xlv., S. 75 and 256.

[2] *Ztschr. f. Heilk.*, 1890, Bd. xi.

[3] Cohnstein and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xlii. S. 303.

0·95 to 1·05 in men, and from ·9 to 1 in women.[1] This "blood quotient" has been also termed "the worth" of a corpuscle.[2]

The more exact methods for the determination of the amount of hæmoglobin in blood are dealt with elsewhere (see article on Hæmoglobin). For clinical purposes a comparison with a standard colour of the colour of the blood diluted to a known amount is found to give sufficiently accurate results. The chief methods used have been—(1) That of Gowers,[3] who employs picrocarmine gelatin as a standard; (2) that of F. Hoppe-Seyler,[4] who combines the hæmoglobin with carbonic oxide, and compares it with a standard solution of CO hæmoglobin; and (3) that of v. Fleischl, who used a wedge of tinted glass as a comparison. The method of v. Fleischl is by far the most convenient. It has been greatly improved by Oliver, who has adapted to it the principle of Lovibond's tintometer.[5] Thus modified it takes the form of a series of tinted glasses, one of which represents accurately the colour of a measured amount of normal blood diluted with water and placed in a flat glass cell of a certain size, whilst the others represent percentages of hæmoglobin below and above the normal (Fig. 22). The blood is measured in a pipette similar to that shown in Fig. 21, *a*.[6]

The number of white corpuscles in a cubic millimetre of blood is usually stated as 10,000, but it varies greatly even in health. By far the larger proportion (70 to 90 per cent.) are of the finely granular oxyphil variety.[7] Of the rest less than 5 per cent. are coarsely granular oxyphil cells, while the remainder, except a few which are hyaline, contain basophil granules.

Injection of many substances (peptones, nuclein, leech extract) into the vessels causes an immediate and marked diminution in the number of the leucocytes, chiefly affecting the finely granular kind (*leucocytopenic phase*, Löwit);[8] it is followed by an increase in their number (*leucocytotic phase*). Acute local inflammation causes similar changes, but the diminution in the number of leucocytes also largely affects the coarsely granular cells, whereas the after increase is mainly in the finely granular. Hankin noticed that the blood clots more readily when the coarsely granular cell is scanty; this may explain the more ready clotting of blood in inflammatory conditions.

The blood possesses, in the presence of free oxygen, a certain power of producing oxidation in readily oxidisable substances, which may be added to it, such as salicylaldehyde.[9] This property it shares with some of the tissues (spleen, liver, lung, thyroid, kidney, thymus), while other tissues show no such tendency (muscle, brain, pancreas). The oxidation power is greater in young subjects than in the adult.[10] On the other hand, the blood contains a substance or substances ("reducing substances" of Pflüger) which greedily appropriate any free oxygen which may be present in the plasma,

[1] Oliver, *loc. cit.*, p. 1705.

[2] Garrod, *Med.-Chir. Trans.*, London, vol. lxxv. p. 191.

[3] *Lancet*, London, 1878, vol. ii. p. 822.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvi. S. 505. See also G. Hoppe-Seyler, *ibid.*, 1896, Bd. xxi. S. 461, and Winternitz, *ibid.*, S. 468.

[5] Lovibond, "Measurement of Light and Colour Sensations."

[6] For more complete details of the method see Oliver, *loc. cit.*, pp. 1699–1703.

[7] Sherrington, *Proc. Roy. Soc. London*, 1894, vol. lv.; Kanthack and Hardy, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvii. p. 81. The earlier literature is given by Sherrington.

[8] "Studien z. Phys. u. Path. d. Blutes," Jena, 1892.

[9] Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vii. S. 115; *Centralbl. f. d. med. Wissensch.*, Berlin, 1892, Bd. xxx. S. 489.

[10] Abelous and Biarnès, *Arch. de physiol. norm. et path.*, Paris, 1895, pp. 195 and 239.

and are even capable of abstracting the oxygen which is combined with hæmoglobin, so that arterial blood rapidly becomes converted into venous blood, when it is not exposed to the access of fresh oxygen. It is not known upon what substance or substances these properties depend, but it is probable that it is a function of the protoplasm of cells, and, in the case of the blood, it may be due to the protoplasm of the white corpuscles.

General composition.—The general composition of blood and the relative distribution of its constituents in the corpuscles and plasma respectively is illustrated in the accompanying tables from C. Schmidt[1] and Bunge.[2]

Venous Blood of a Man, æt. 25, sp. gr. 1·0599 (C. Schmidt).

In 1000 grms. blood corpuscles (sp. gr. 1·0886)—		
Water		681·63
Substances not vaporising at 120° C.		318·37
Hæmoglobin and other proteid substances		311·09
Inorganic substances—		
Chlorine	1·750	
Sulphuric acid	0·061	
Phosphoric acid	1·355	
Potassium	3·091	
Sodium	0·470	
Phosph. lime	0·094	
Phosphate magnesia	0·060	
Oxygen	0·401	
Total of inorganic constituents (exclusive of iron)	———	7·282
In 1000 grms. plasma (sp. gr. 1·0312)—		
Water		901·51
Substances not vaporising at 120° C.		98·49
Fibrin		8·06
Other proteids and organic substances		81·92
Inorganic substances—		
Chlorine	3·536	
Sulphuric acid	0·129	
Phosphoric acid	0·145	
Potassium	0·314	
Sodium	3·410	
Phosphate lime	0·298	
Phosphate magnesia	0.218	
Oxygen	0·455	
Total of inorganic constituents	———	8·505

In this estimation the phosphoric acid is probably too high, being increased in the process of calcining by the phosphorus in the lecithin. Sertoli,[3] by eliminating this error, obtained only 0·025 grm. phosphoric acid per 1000 grms. ox serum, equivalent to only 0·005 per cent. hydrodisodic phosphate (Na_2HPO_4).

It is clear from the following table that there are considerable differences in the composition of the whole blood and of its parts in

[1] "Charakter. der epid. Cholera," Leipzig, 1850.

[2] *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 191; and "Physiol. and Pathol. Chemistry," trans. by Wooldridge, 1890, p. 245.

[3] Sertoli in *Hoppe-Seyler's Med. Chem. Untersuch.*, Berlin, 1868, S. 352. See also Miroczkowski, *Centralbl. f. d. med. Wissensch.*, Berlin, 1878, S. 353, who obtained in calf serum, 0·018; in sheep serum, 0.0092 and 0·0064; and in dog serum, 0·0083 parts Na_2HPO_4 per 100 serum.

different species of animals. In most animals the blood corpuscles have a relatively large proportion of potash salts and phosphates, whereas the preponderating salt in the serum is sodium chloride. In the bullock, however, this salt also occurs in large amount in the corpuscles.

Defibrinated Blood of Pig, Horse, and Bullock (Bunge).

	In 1000 Grms. Corpuscles.			In 1000 Grms. Serum.		
	Pig.	Horse.	Bullock.	Pig.	Horse.	Bullock.
Water	632·1	608·9	599·9	919·6	896·6	913·3
Solids	367·9	391·1	400·1	80·4	103·4	86·7
Proteids, including hæmoglobin	347·1	...	387·8	67·7	...	73·2
Other organic substances	12·0	...	7·5	5	...	5·6
Inorganic substances	8·9	...	4·8	7·7	...	7·9
K_2O	5·543	4·92	0·747	0·273	0·27	0·254
Na_2O	...	...	2·092	4·272	4·43	4·351
CaO	...	...	...	0·136	...	0·126
MgO	0·158	...	0·017	0·038	...	0·045
Fe_2O_3	...	...	...	...	...	0·011
Cl	1·504	1·93	1·635	3·611	3·75	3·717
P_2O_5	2·067	...	0·703	0·188	...	0·266

In the pig's blood analysed, there were, in 1000 parts, 436·8 corpuscles, and 563·2 serum.

In the horse's blood analysed, there were, in 1000 parts, 531·5 corpuscles, and 468·5 serum.

In the bullock's blood analysed, there were, in 1000 parts, 318·7 corpuscles, and 681·3 serum.

A. Schmidt, in conjunction with his pupils,[1] got the following results from analyses of human blood obtained by venesection.

	Percentage Amount.	Specific Gravity.	Amount of Dry Residue in 100 Grms.	Amount of Na, K, and Cl, in 100 grms. (Warnach).		
				Na.	K.	Cl.
Serum	52·120	1028·3	9·709	0·344	0·02	0·353
Corpuscles	47·880	...	35·458	0·282	0·307	...
Total blood	...	1060·7	21·971	0·185	0·182	0·259

Gases of the blood.—Arterial blood of the dog contains from 15 to 25 vols. per cent. oxygen (at 0° C. and 760 mm. pressure), 25 to 40 carbonic anhydride, and about 1·8 vols. per cent. nitrogen. Venous blood of the same animal contains from 5 to 15 vols. per cent. oxygen, 38 to 52 carbonic anhydride, and also about 1·8 vols. per cent. nitrogen.[2] The proportions of these gases and the manner in which they are combined with constituents of the corpuscles and plasma is discussed elsewhere.[3]

[1] Arronet, Diss., Dorpat, 1887 ; Warnach, Diss., Dorpat, 1888.

[2] Schöffer, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1860, Bd. xli. S. 589 ; Sczelkow, *ibid.*, 1862, Bd. xlv. S. 171 ; Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 275.

[3] See "Chemistry of Respiration."

The red corpuscles.—These consist of a delicate external envelope enclosing coloured fluid contents.[1] In all vertebrates below mammals they contain a nucleus, the chief chemical constituent of which is nuclein (see p. 65).

The organic matter in one hundred parts of dried red corpuscles consists of:[2]—

	Human Blood.		Dog's Blood.	Goose's Blood.
	II.	I.		
Proteids and nuclein . .	12·24	5·10	12·55	36·11
Hæmoglobin .	86·79	94·30	86·50	62·65
Lecithin . .	0·72	0·35	0·59	0·46
Cholesterin .	0·25	0·25	0·36	0.48

Goose's blood was taken as an instance of one in which nucleated red corpuscles are present; the higher percentage of proteids apparent in this is due to the included nuclein.

The mineral constituents of the red corpuscles vary greatly in relative quantity in different species of animals. Thus potassium constitutes 40·89 per cent. of the total ash of human red corpuscles, and sodium only 9·71, whereas in the dog the percentage of potassium is 6·07, and of sodium 36·17 (C. Schmidt).

The remarkable excess of potassium over sodium salts is the opposite to their relative proportion in plasma.

The chief organic constituent of the corpuscles, hæmoglobin, will be considered in a separate article. The other organic constituents consist of nucleo-proteid, lecithin, and cholesterin.

The nucleo-proteid of the red corpuscle.—Wooldridge's[3] method for obtaining the nucleo-proteid consists in centrifugalising defibrinated blood repeatedly with a 1 per cent. sodium chloride solution until all the serum is washed away. The red corpuscles are then laked by the addition of water, and the mixture is shaken with a little ether, to assist the solution; the white corpuscles are allowed to settle, or removed by the centrifuge. To the clear but highly coloured decanted fluid a little 1 per cent. solution of acid sodium sulphate is added. This causes a considerable precipitate of nucleo-proteid, which is chiefly derived from the red corpuscles, but a small part of which may come from the white corpuscles and blood platelets.

The material thus obtained was shown by Kühne,[4] who used a rather different method of separating it, to possess fibrino-plastic properties. It was further examined by Halliburton and Friend,[5] who found that it was

[1] Schäfer in Quain's "Anatomy," 10th edition, 1893, vol. i. pt. 2, p. 210.

[2] Hoppe-Seyler and Jüdell, *Med. Chem. Untersuch.*, Berlin, 1866, Heft 3. Manasse, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 452, gives the following percentages—Lecithin, 1·687; Cholesterin, 0·151.

[3] *Arch. f. Physiol.*, Leipzig, 1881, S. 387.

[4] "Lehrbuch," S. 193.

[5] *Journ. Physiol.*, Cambridge and London, 1886, vol. x. p. 532.

identical in properties with what had been called by Halliburton cell globulin-β (see p. 82). When cell globulin-β was discovered to be a nucleo-proteid, this also was found to be of the same nature.[1] It can also be prepared from the red corpuscles by Wooldridge's acetic-acid method, or, provided the corpuscles are well caked together by the action of an efficient centrifuge, by the sodium-chloride method of Halliburton. In these experiments the colourless corpuscles may be got rid of by a previous injection of commercial peptone.

In cats the percentage of phosphorus in the corpuscular nucleo-proteid is 0·68. It produces intravascular coagulation when a solution in 1 per cent. sodium carbonate is injected intravenously (Halliburton).

The lecithin and cholesterin. — L. Hermann[2] and Hoppe-Seyler[3] described the phosphorus-containing organic constituent of the corpuscle as protagon, a substance got in large quantities from medullated nerves, but subsequently it was recognised by Hoppe-Seyler[4] to be in reality lecithin, which is a decomposition product of protagon (see p. 83). Both lecithin and cholesterin are extracted from the corpuscles by ether, and are therefore either free or, at most, in very loose combination with the nucleo-proteid.

The chemical composition of the white corpuscles has been already dealt with (p. 83).

The blood platelets.—In spite of the large amount of research from the histological standpoint which has been carried out in relation to the blood platelets (*Blutplättchen* of Bizzozero), very little is known about their function or their chemical composition. According to Löwit,[5] they consist chiefly of a globulin, and play an important part in fibrin formation. As the result of microchemical work, Lilienfeld[6] considers that they consist of nucleo-proteid.

Löwit states that they are not to be seen in the circulating blood,[7] and regards them as being produced partly from the white corpuscles, partly from globulins of the plasma, after withdrawal of the blood. They can, however, be seen within capillary blood vessels which have just been removed from animals, and in which the blood is still fluid.[8] Mosen failed to find them in lymph.[9]

Their number in the blood has been variously estimated at from 180,000 to over 600,000 per c.mm.[10]

Blood Plasma.

The methods of obtaining plasma from blood, by preventing coagulation and allowing the corpuscles to subside, have already been given. Obtained thus from a suspended vein or from a cooled vessel, plasma

[1] Halliburton, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 306.
[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1866, S. 33.
[3] *Med. Chem. Untersuch.*, Berlin, Heft 1, S. 140.
[4] *Ibid.*, Heft 3.
[5] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1888, Bd. xxiv. S. 188.
[6] *Arch. f. Physiol.*, Leipzig, 1892, S. 115.
[7] *Virchow's Archiv*, 1889, Bd. cxvii. S. 545 ; and "Studien z. Phys. u. Path. d. Blutes u. d. Lymphe," Jena, 1892.
[8] Osler, *Proc. Roy. Soc. London*, 1874, No. 183. This observation I can entirely confirm.
[9] *Arch. f. Physiol.*, Leipzig, 1893, S. 352. See also Druebin, 1892, *ibid.*, Suppl., S. 211.
[10] See on this subject, Muir, *Journ. Anat. and Physiol.*, London, 1891, vol. xxi. ; also Brodie and Russell, *Journ. Physiol.*, 1897, vol. xxi. p. 390, who give reasons for regarding the higher number as more correct. Probably, however, the number varies greatly.

is a clear yellowish liquid of alkaline reaction and sp. gr. about 1027–1031. It contains about 90 per cent. of water, holding various organic and inorganic substances in solution. With the exception of certain proteids, the constituents of plasma are identical with those of serum, in which they are more readily studied.

Inorganic substances.—Plasma consists to about 90 per cent. of water. The inorganic salts occur to the amount of about 0·8 per cent. The principal is chloride of sodium. This can be crystallised out from plasma after inspissation. According to the analyses of C. Schmidt, it is present to the extent of 0·55 per cent. Carbonate of soda is probably the next most abundant salt, although its exact amount cannot be stated. It is to this salt that plasma mainly owes its alkalinity and its power of absorbing carbonic acid. Although it is not possible to state definitely in what manner the acids and bases of the plasma are distributed, it appears probable that, besides these two salts, chloride of potassium, sulphate of potassium, phosphate of calcium, phosphate of sodium, and phosphate of magnesium, and probably chloride of calcium, occur[1] in small amounts. Traces of a fluoride have also been found.[2]

Gases.—The gases of plasma have not been satisfactorily investigated. They are probably not very different from those of serum, which in the dog consist of from 43 to 57 vols. of carbonic anhydride, 2·25 of nitrogen, and 0·25 of oxygen.[3] The oxygen and nitrogen are probably simply dissolved in the plasma, but the carbonic anhydride is present in far too great an amount for this to be the case, since not more than 2 or 3 vols. per cent. of this gas could be dissolved. The remaining amount must therefore be in chemical combination. This can only be with soda, as carbonate and bicarbonate; for other bases are present in too small amount in plasma to be taken into serious consideration. This statement is also true for alkaline phosphates, although in the corpuscles, in which they are present in considerable quantity, they may play an important part in fixing CO_2 (Bunge), as shown by the following equation:—

$$Na_2HPO_4 + H_3CO_3 = H_2PO_4 + NaHCO_3.$$

Some of the CO_2 may be combined with proteid,[4] but this can only be very little. As a matter of fact, Bunge calculates that, after allowing for the amount of soda required to saturate the only strong mineral acid of the plasma (hydrochloric), there is enough left to fix 63 vols. per cent. of CO_2 as carbonate, and an equal additional amount as bicarbonate, which is far more than the amount of CO_2 actually present.[5]

Organic constituents of blood plasma.—The organic constituents of plasma may be divided into proteids and non-proteids, and the latter into nitrogenous and non-nitrogenous.

Non-nitrogenous organic substances found in plasma.—These consist of carbohydrates and fats; and, in addition, there are present small quantities of a lipochrome, of cholesterin, and probably of sarcolactic acid.

Carbohydrates of plasma.—Three carbohydrates have been described

[1] Pribram, *Abhandl. d. math.-phys. Cl. d. k. Sächs. Gesellsch. d. Wissensch.*, Math.-phys. Klasse, 1871, Bd. xxiii. S. 279; and in *Arb. a. d. physiol. Anst. zu Leipzig*, 1871, p. 63.

[2] Tammann, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 325.

[3] Bunge, *op. cit.*, S. 286.

[4] Sertoli, *Hoppe-Seyler's Med. Chem. Untersuch.*, Berlin, 1868, Heft 3, S. 350.

[5] *Op. cit.*, S. 286.

in plasma, namely—(1) glycogen; (2) an animal gum; (3) dextrose or grape sugar.

1. *Glycogen.*—There seems to be no doubt that traces of glycogen can be obtained from fresh blood. Some is said to occur free in plasma, but if so it is probably derived from intermixed or disintegrated leucocytes, which can be shown by histochemical reaction to contain it.[1] Kaufmann finds the amount of glycogen in blood to be greatly increased (from 0·025 to 0·59 per litre) by removal of the pancreas.[2]

2. *Animal gum.*—Freund[3] has obtained from blood a carbohydrate substance, resembling that described by Landwehr under the above name. It has the formula $(C_6H_{10}O_5)_n$, and is converted by boiling with dilute mineral acids into a substance (sugar) which reduces Fehling's solution, but is not fermentable, nor is it rotatory for polarised light. Four litres of ox blood yielded 0·82 grms. of the gum, giving a percentage amount of 0·02.

3. *Dextrose.*—This is a constant constituent of plasma, whatever the nature of the diet, and even in starving animals.[4] It occurs in man to the amount of about 0·12 per cent. of the blood, in the dog from 0·11 to 0·15 per cent. (or a little over 1 per 1000).[5] It is present in nearly equal amount in blood from all parts, except in the blood of the portal vein, during digestion of carbohydrate-containing foods, where it is markedly increased. In the blood of the hepatic veins, in the intervals of digestion, the amount was stated by Bernard to be somewhat greater than in the portal vein, or in the blood of the general circulation; but this difference has not been found by Pavy and most other observers, although the statement has of late been reaffirmed by Seegen.[6]

Bernard[7] obtained a larger amount of sugar from arterial than from venous blood, and Seegen has in some instances obtained a similar result. Chauveau,[8] and Chauveau and Kaufmann,[9] have also published analyses, which seem to show a disappearance of sugar after passing the capillaries. But the differences observed have not been constant, and are in any case so small as to lie within the range of experimental error. As the result of eleven experiments, Pavy finds the sugar in arterial blood to exceed that in venous by only 0·003 parts per 1000; and he concludes that no appreciable difference exists between the two.[10]

[1] E. A. Schäfer, "A Course of Practical Histology," London, 1876, p. 39; Salomon, *Deutsche med. Wchnschr.*, Leipzig, 1877, S. 92 and 421; *Arch. f. Physiol.*, Leipzig, 1878; *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892, Bd. vi. S. 512; Ehrlich, *Ztschr. f. klin. Med.*, Berlin, 1883, Bd. vi. S. 40; Gabritschewsky, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 272; Huppert, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892, No. 14, S. 394 (Huppert found more in dog's blood than in the blood of herbivora); Hoppe-Seyler, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xviii. S. 144.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1895, tome cxx. p. 567.

[3] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892, Bd. vi. S. 345.

[4] Cl. Bernard, *Arch. gén. de méd.*, Paris, 1848, tome xviii. p. 303; Pavy, *Phil. Trans.*, London, 1860; v. Mering, *Arch. f. Physiol.*, Leipzig, 1877, S. 379; Otto, *Arch. f. d. ges. Physiol.*, Bonn, 1885, Bd. xxxv. S. 467; Pickardt, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xvii. S. 217; Miura, *Ztschr. f. Biol.*, München, Bd. xxxii. S. 255.

[5] Pavy, "Physiology of the Carbohydrates," 1894, p. 161.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiv. S. 388, and 1885, Bd. xxxvii. S. 348; *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, No. 12; "Zuckerbildung im Thierkörper," 1890.

[7] *Compt. rend. Acad. d. sc.*, Paris, tome lxxxiii. p. 373, and "Leçons sur le Diabète," 1877.

[8] *Ibid.*, 1856, tome xliii. p. 1008.

[9] *Ibid.*, 1886, tome ciii. p. 974.

[10] Pavy, *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 346; "On Certain Points connected with Diabetes," London, 1878; "Physiology of the Carbohydrates," pp. 170–171. This is also apparently admitted by Seegen ("La Glycogenie Animale," Paris, 1890, p. 100), although his theory of the production of energy requires that there should be a diminution in the amount of sugar in venous blood.

Apart from these somewhat doubtful differences in blood from different parts, the amount in the blood remains almost constant, whatever the character of the food, and even during starvation. The amount is somewhat increased as the result of hæmorrhage, a result due either to accession of lymph (which contains a larger proportion of sugar than does blood), or to the operation, through the agency of the nervous system, causing an increased production of sugar from the liver-glycogen. If the amount of dextrose in the blood be artificially increased to more than about 0·25 per cent. the excess passes off by the urine. The amount is increased in diabetes, whether this be the result of the sugar puncture, of removal of pancreas, or of disease,[1] but even under these circumstances does not rise above 0·48 per cent.

Fats.—These are present in plasma in small but variable quantity (0·2 to 0·5 or even 1 per cent.)[2] being most abundant after a meal containing much fat. The plasma or serum may then be milky from admixture with the fat-containing chyle. They are composed of the usual glycerides of fatty acids (palmitin, stearin, and olein). A small amount, 0·05–0·1 per cent., is in the form of soap.[3] It has been stated[4] that there is a greater amount of fat (ether extract) in arterial than in venous blood, but this result is shown by Röhmann and Mühsam[5] to have been probably due to an error brought about by venous congestion, which affects the proportion of all the solids of blood as compared with the water. The fatty acids appear also to be partly in combination with cholesterin, forming cholesterin-esters, of which two have been separated by Hürthle[6] in a crystalline form, namely, the olein and palmitin compounds, to the extent in horse serum of 0·08 and 0·06 per cent. respectively. Hürthle further found that in the dog they were increased during inanition. The amount of cholesterin in serum or plasma is stated by Hoppe-Seyler to be about 0·05 gr. per 100 c.c. blood,[7] and is probably mainly in the form of the fatty acid combinations just referred to, and not, as was formerly supposed, in the free condition (Hürthle).

Lipochrome.—The yellow-colouring matter of serum is a lipochrome soluble in amylic and also in ethylic alcohol, but insoluble in turpentine. Its absorption spectrum shows two ill-defined bands,[8] one at the F and the other between the F and G Frauenhofer lines (Plate III., Fig. 24). It resembles the lutein of Kühne.

Lactic acid.—The presence of sarcolactic acid as a regular constituent of normal blood plasma has been affirmed (0·017–0·054 per cent. in dogs).[9] Salomon could only find it in blood from the dead body, not in that drawn during life,[10] but Irisawa confirms its existence in fresh blood (dog), and states that it is present to some extent in the corpuscles as well as in the plasma.[11] It is increased in blood which has

[1] Pavy, "On Certain Points connected with Diabetes"; Seegen, *Wien. med. Wchnschr.*, 1886, S. 1561 and 1595.

[2] Röhrig, *Abhandl. d. math.-phys. Cl. d. k. Sächs. Gesellsch. d. Wissensch.*, 1874, S. 1, and *Arb. a. d. physiol. Anst. zu Leipzig.*

[3] Hoppe-Seyler, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. viii. S. 503.

[4] Bornstein, Diss., Breslau, 1887.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xlvi. S. 383.

[6] *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 331.

[7] *Med. Chem. Untersuch.*, Berlin, 1866, S. 145.

[8] Krukenberg, *Sitzungsb. d. Jenaisch. Gesellsch. f. Med. u. Naturw.*, 1885, Suppl. Bd. xix. S. 25.

[9] Gaglio (with Drechsel), *Arch. f. Physiol.*, Leipzig, 1886, S. 400; Spiro, *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. i. S. 110; Berlinerblau (with Nencki), *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxiii. S. 333.

[10] *Virchow's Archiv*, 1888, Bd. cxiii. S. 356.

[11] *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xvii. S. 340.

been perfused through the still living kidneys or lungs, or through the muscles of the lower limb, especially if inosit or glycogen or dextrose be added to the blood used for perfusion (Gaglio, Berlinerblau). It is also increased by intravenous injection of dextrose in blood circulating normally through the body. It appears to enter into combination with sodium hydrate, driving out CO_2.[1]

Non-proteid nitrogenous constituents of plasma.—The most important of these are urea[2] (0·02–0·05 per cent.), kreatin, kreatinine,[3] and uric acid,[4] and occasionally hippuric acid.[5] Xanthine and hypoxanthine are stated to be also present.[6] Gréhant and Quinquand found the amount of urea in blood drawn from the splenic, portal, and hepatic veins to be slightly greater than in that taken from the carotid.[7] Lecithin occurs in small amount in plasma.[8] According to Marino-Zucco, neurine and glycero-phosphoric acid are also present in traces in the free state. There has also been described as a constant constituent, jecorin[9]—a substance which reduces Fehling's solution, but is soluble in ether and is not fermentable. It is stated to occur in considerably larger amount in venous than in arterial blood.[10]

Ferments.—Three ferments have been described as occurring in blood, namely—

1. A diastatic ferment, producing the conversion of amyloids to sugar.
2. A glycolytic ferment, producing the disappearance of sugar.
3. A fat-splitting ferment (lipase).[11]
4. A fibrin ferment (thrombin), or its precursor (prothrombin), producing the formation of fibrin from fibrinogen. The last will be considered in connection with coagulation.

Diastatic action.—A ferment action, converting starch into dextrin and maltose, and ultimately into dextrose, has been obtained with blood and lymph by Röhmann[12] and Bial,[13] and also by Hamburger,[14] by

[1] Vaughan Harley, *Arch. f. Physiol.*, Leipzig, 1894, S. 451.

[2] Simon, *Arch. f. Anat. u. Physiol.*, Leipzig, 1841, S. 454; I. Munk, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. xi. S. 105; Schröder, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1882, Bd. xv. S. 364; and 1885, Bd. xix. S. 373. Picard (*Journ. de l'anat. et physiol. etc.*, Paris, 1881, p. 530) found the percentage of urea rather higher than this in the dog (0·09 to 0·13).

[3] Verdeil and Marcet found both kreatin and kreatinine (*Journ. de pharm. et chim.*, Paris, 1851, tome xx. p. 89); Voit (*Ztschr. f. Biol.*, München, 1868, S. 93) could find no kreatinine; but Colls (*Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 107) obtained a small but definite quantity.

[4] Scherer and Strecker, quoted by Hoppe-Seyler ("Physiol. Chem."); Garrod, *Med.-Chir. Trans.*, London, 1848, vol. xxxv. p. 83, and 1854, vol. xxxvii. p. 49. See also "Nature and Treatment of Gout," 1861; Abèles, *Med. Jahrb.*, Wien, 1887, S. 479. On the other hand, v. Jaksch (*Ztschr. f. Heilk.*, 1890, Bd. xi. S. 415) could find no uric acid in the blood of healthy individuals (nine cases).

[5] Verdeil and Goldfuss, *Compt. rend. Soc. de biol.*, Paris, 1850, tome ii. p. 79. Meissner and Shepard ("Untersuch. ü. d. Ensteh. d. Hippurs.," Hannover, 1866) were unable to find it.

[6] Halliburton, "Chem. Physiol.," p. 251.

[7] *Journ. de l'anat. et physiol. etc.*, Paris, 1884, p. 317.

[8] Hoppe-Seyler, *Med. Chem. Untersuch.*, Berlin, 1869, S. 551.

[9] Baldi, *Arch. f. Physiol.*, Leipzig, 1887, Suppl. Heft, S. 100; Henriques, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxiii. S. 244.

[10] Jacobsen, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892, S. 368.

[11] Hanriot, *Compt. rend. Soc. de biol.*, Paris, 1896, p. 925.

[12] *Arch. f. d. ges. Physiol.*, Bonn, 1892, Bd. lii. S. 157.

[13] *Ibid.*, 1892, Bd. lii. S. 137; and Bd. liii. S. 156; Röhmann and Bial, *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 72; Bd. lv. S. 469. According to Lépine and Barral (*Compt. rend. Acad. d. sc.*, Paris, 1893, tome cxiii., pp. 118, 729, 1014, and cxv. p. 304) sugar may be formed in blood on standing, at the expense of added peptone, as well as starch or glycogen; but this was not confirmed by Bial.

[14] *Ibid.*, 1895, Bd. lx. S. 543.

mixing blood or serum with starch or glycogen solution, and keeping it at body temperature. Röhmann has shown that the diastatic change may occur in lymph within the vessels as well as *in vitro*. Cavazzani obtained most effect in blood taken from the portal vein.[1] Tscherevkoff finds that the diastatic ferment is precipitated by excess of alcohol, and that its action is not destroyed by long standing under alcohol, nor by sodium oxalate.[2]

Glycolytic action.—It was noticed by Bernard[3] that the sugar of blood diminished on standing *in vitro*. Pavy found that both the normal sugar and added sugar diminishes in blood on standing.[4] In any case, and without standing, it is difficult to recover the full amount from blood or serum, apparently owing to the fact that, in coagulating the proteids with a view to their removal, a part of the sugar is mechanically carried down or retained by them;[5] this fact may lead to very considerable experimental errors.[6] Allowing, however, for such errors, it appears clear that there is some actual loss of sugar on standing both in blood[7] and in lymph or chyle.[8] According to Seegen, the glycolytic action is active in the presence of chloroform, and is destroyed by a temperature of more than 54° C., in these respects resembling an enzyme. Lépine states that it is absent or diminished in activity in diabetes,[9] whether the result of disease or operation (removal of pancreas), and that a very active glycolysis occurs in perfusing blood through various organs (kidney, lower limbs).[10] Arthus, on the other hand, denies the pre-existence of a glycolytic ferment in blood. He finds no glycolysis in oxalated blood, and thinks it probable that the ferment is formed from leucocytes during coagulation.[11] Kraus finds that the glycolysis which occurs in blood on standing is accompanied by a splitting off of CO_2, and is probably due therefore to oxidation.[12]

Proteids of plasma.—The proteids of plasma are—

1. One or more closely allied albumins (serum albumins).
2. Two globulins, termed respectively serum globulin and fibrinogen.
3. A nucleo-proteid or nucleo-proteids.

Blood contains normally neither albumose nor peptones.[13] All the proteids are completely precipitated by saturating plasma with ammo-

[1] *Arch. per le sc. med.*, Torino, 1893, vol. xvii. p. 105.
[2] *Arch. de physiol. norm. et path.*, Paris, 1895, p. 628.
[3] *Compt. rend. Acad. d. sc.*, Paris, 1876, p. 1406.
[4] *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 346; and 1879, vol. xxvii. p. 520. See also "Physiol. of Carbohydrates," pp. 171–179.
[5] Röhmann, *Centralbl. f. Physiol.*, Liepzig u. Wien, 1890, No. 1; V. Harley, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 391; Pavy, *Brit. Med. Journ.*, London, 1896, vol. i. p. 453.
[6] Schenck, *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 607; 1891, Bd. xlvii. S. 621. For a method whereby such errors may be largely avoided see E. Waymouth Reid, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 316.
[7] Röhmann, *loc. cit.*; Harley, *loc. cit.*; Seegen, *Wien. klin. Wchnschr.*, 1892, Nos. 14 and 15.
[8] Lépine, *Compt. rend. Acad. d. sc.*, Paris, 1890, tome cx. p. 742; Lépine and Barral, *ibid.*, 1890, tome cx. p. 134; *ibid.*, 1891, tome cxii. pp. 411, 604, 1185, 1414; and tome cxiii. p. 118.
[9] Lépine and Metroz, *ibid.*, 1893, tome cxvii. p. 154.
[10] Lépine and Barral, *loc. cit.*
[11] *Arch. de physiol. norm. et path.*, Paris, 1892, p. 337; *Compt. rend. Acad. d. sc.*, Paris, 1892, tome cxiv. p. 605.
[12] *Ztschr. f. klin. Med.*, Berlin, 1892, Bd. xxi. S. 315. See also Röhmann and Spitzer, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. xxviii.; and Spitzer, *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lx.
[13] Halliburton and Colls, *Journ. Path. and Bacteriol.*, Edin. and London, 1895, p. 295.

nium sulphate. The globulins and nucleo-proteids are completely precipitated by half-saturation with ammonium sulphate, or by complete saturation with magnesium sulphate; whilst fibrinogen is precipitated by half-saturating plasma with chloride of sodium (probably some nucleo-proteid is carried down with it). Upon these differences of solubility in solutions of neutral salts the separation of the blood-proteids one from another depends.

The proportion of globulin to albumin $\frac{\text{globulin}}{\text{albumin}}$ is known as the "proteid quotient"; it varies in different animals and in the same species of animal under different conditions.[1] For the same individual it is almost constant in the blood serum, lymph, and serous transudations, although the absolute amount of proteid in these may vary greatly.[2]

The annexed table[3] shows the total and relative amounts of the proteids in the serum of different animals. The numbers are taken from different sources; the first four from Hammarsten.[4]

They are obtained—(*a*) the total proteids, by weighing the alcohol precipitate; (*b*) the globulin, by separating off the magnesium sulphate precipitate, re-dissolving this and weighing its alcohol precipitate; (*c*) the albumins, by taking the difference between these two results. (*b*) includes, besides serum globulin, a globulin formed from fibrinogen in coagulation, and also the nucleo-proteids of plasma, but both of these are in very small amount.

	(*a*) Total Proteids per Cent.	(*b*) Globulins per Cent.	(*c*) Albumins per Cent.
Man	7·62	3·10	4·52
Horse	7·25	4·56	2.69
Ox	7·50	4·17	3·33
Rabbit	6·22	1·79	4·43
Pigeon	5·01	1·32	3·69
Hen	4·14	2·90	1·24
Tortoise	4·76	2·82	1·94
Lizard	5·16	3·33	1·83
Terrapin	5·35	4·66	0·69
Snake	5·32	4·95	0·37
Frog	2·54	2·18	0·36
Toad	3·22	1·82	1·40
Newt	3·74	3·31	0·43
Eel	6·73	5·28	1·45
Dog-fish	1·62	1·17	0·45

The most noteworthy feature shown in these figures is the relatively small amount of albumins present in the serum of cold-blooded animals as compared with the globulins. It has been stated that the albumins proportionately diminish in starved animals,[5] but other investigators have failed to confirm this conclusion.[6]

[1] Compare Frassineto, *Arch. ital. de biol.*, Turin, 1895, vol. xxiv. p. 457; Paulesco, *Arch. de physiol. norm. et path.*, Paris, 1897, p. 21; W. Engel, *Arch. f. Hyg.*, München u. Leipzig (4), Bd. xxviii. S. 334.

[2] Salvioli, *Arch. f. Physiol.*, Leipzig, 1881, S. 269; Hoffmann, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1882, Bd. xvi. S. 133.

[3] Halliburton, *Journ. Physiol.*, Cambridge and London, 1878, vol. vii. p. 321.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 413.

[5] Tiegel, *ibid.*, 1880, Bd. xxiii. S. 278; Burckhardt, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1883, Bd. xvi. S. 322.

[6] Salvioli, *Arch. f. Physiol.*, Leipzig, 1881, S. 269; Howell, *John Hopkins Univ. Stud. biol. lab.*, Baltimore, vol. iii. p. 49; Rubbrecht, *Trav. du lab. de L. Fredericq* tome v. p. 121.

Albumins of blood plasma.—The albumins of plasma are also found in the serum after coagulation of blood, and hence they have been termed serum albumins. They remain in plasma or serum after half-saturating it with ammonium sulphate, *i.e.* by mixing it with an equal amount of saturated ammonium sulphate solution, or after entirely saturating it with magnesium sulphate.

The precipitated globulins and nucleo-proteid are removed by filtration, and the filtrate dialysed to remove the salts. The solution which remains contains only the albumins; they can be precipitated from it by saturation with ammonium sulphate or by sodio-magnesium sulphate. According to Gürber, they can be obtained in a crystalline form by adding ammonium sulphate just sufficient to produce precipitation and allowing the fluid to stand exposed to the air.[1]

The material obtained in these ways constitutes what has usually been called serum albumin (serine), but, as Halliburton has shown,[2] it is really a mixture of three separate albumins, which he has termed respectively α, β, γ. These differ from one another in their temperature of heat coagulation; α-albumin coagulates at 72°–75° C.; β-albumin at 77°–78° C.; and γ-albumin at 83°–86° C. In the plasma of horse, ox, and sheep blood, α-albumin is absent, but the other two are present; in man, and all other mammals and birds investigated by Halliburton, all three were present; but in reptiles, amphibia, and fishes investigated, α-albumin was usually the only one found.[3]

The crystals of serum albumin which were obtained by Gürber from the serum of horse's blood were hexagonal prisms with one pyramidal end, and were doubly refracting; some of them were as much as 1 cm. long. Their elementary composition was C, 53·1; H, 7·1; N, 15·9; S, 1·9, 0·22; and ash, 0·22 per cent. Dissolved in water and the excess of ammonium sulphate removed by dialysis, the solution had a heat coagulation temperature of 51° to 53°, and a specific rotation for yellow light of −61°.[4]

The globulins of blood plasma.—The globulins of blood plasma consist of serum globulin and fibrinogen. Serum globulin (paraglobulin, Kühne; fibrino-plastic substance, A. Schmidt) has a heat coagulation temperature of 75° C., which is almost constant in all animals in which it has been examined. The amount to which it is contained in plasma is represented by the figures in the second column of the table on p. 162. It will be seen from this, that in man it constitutes about three parts per cent. of the total serum. It is precipitated from serum by half-saturation with ammonium sulphate,[5] and also by complete saturation with magnesium sulphate, sodium chloride, and some other neutral salts which do not precipitate the albumins; also, but less completely, by diluting the serum with water (fifteen times) and passing CO_2 through it, or by

[1] *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1894, S. 143.

[2] *Journ. Physiol.*, Cambridge and London, vol. v. p. 152.

[3] In the slider terrapin (Howell, *John Hopkins Univ. Stud. biol. lab.*, Baltimore, vol. iii. p. 49) the albumin present is apparently of the β variety, coagulating at 77° to 80°, and in the eel and dog-fish this variety was also found by Halliburton (*Journ. Physiol.*, Cambridge and London, vol. vii. p. 320).

[4] Michel (with Gürber), *Verhandl. d. phys.-med. Gesellsch. zu Würzburg*, 1895, N. F., Bd. xxix. No. 3.

[5] Kauder (*Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xx. S. 411) found that solutions of ammonium sulphate stronger than 24 per cent. completely precipitated serum globulin; above 33·5 per cent. some of the serum albumin also comes down. A half-saturated solution contains about 26 per cent.

diluting with water and neutralising it with dilute acetic acid (in excess of which it easily dissolves). Like other globulins, it requires the presence of a certain amount of salts, or weak alkali, to be dissolved in water; it is therefore precipitated by dialysis or by sufficient dilution of its solutions in salts or in serum, even without the addition of an acid.

Fibrinogen.—This is the substance to which the plasma of the blood especially owes its property of so-called spontaneous coagulability; which led to the term "coagulable lymph" being applied to it by older writers.[1] It is precipitated from plasma along with serum globulin, by saturation with magnesium sulphate or sodium chloride; the precipitation of mixed globulins so obtained (the *plasmine* of Denis) forms a coagulable liquid, on dissolving it in a more dilute solution of salt. Fibrinogen is entirely precipitated from plasma, or any other fluid containing it, by half-saturation with sodium chloride;[2] it can be re-dissolved in water with the aid of the salt adhering to it, reprecipitated by half-saturation, and so on until it is obtained in a condition which may be regarded as approaching purity. But in contact with the salt solution it gradually loses its solubility, and every time that it is precipitated less of the precipitate redissolves on adding water; the material which forms and which remains undissolved in the dilute solution of salt resembles fibrin in many physical and chemical characters, but is not similarly rapidly swollen by dilute acids; it may be termed *para-fibrinogen* or *pseudo-fibrin.* Fibrinogen dissolves also in dilute alkali, even in the absence of neutral salts; its alkaline solutions are clear, but its solutions in neutral salt solutions are opalescent. It is precipitated from the solution in weak alkali by careful neutralisation with acetic acid, and from solutions in neutral salt solutions by slightly acidulating with the same acid, but it is readily soluble in excess of the acid. The temperature of heat coagulation of fibrinogen in salt solution is between 52° and 55°;[3] but the whole of the dissolved proteid is not thrown down at this temperature; a small amount remains in solution, and is not coagulated until the temperature of 65° C. is attained. According to Hammarsten,[4] this is due to the splitting of the fibrinogen, under the influence of heat, into coagulated fibrinogen and a globulin, which is coagulated at the higher temperature. If fibrinogen which has been obtained from blood plasma by the above method of half-saturation with NaCl, and purified by repeated re-solution and re-precipitation with acetic acid, be dissolved in water rendered faintly alkaline by NaHO, it gives a coagulum-like precipitate (if sufficiently concentrated) a short time after the addition of a lime salt. The coagulum resembles fibrin in many respects, but, according to Hammarsten, it is not true fibrin, but a combination of fibrinogen with lime.[5]

[1] Houlston, Diss. Med. Inaug., "de Inflammatione," pp. 11, 12, 14, Lugd. Bat., 1767. See Hewson's Works, Introduction, p. xxxvii, edited by G. Gulliver, London, printed for the Sydenham Society, 1846.

[2] Hammarsten, *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix. S. 563.

[3] Hammarsten, *ibid.*, 1880, Bd. xxii. S. 431.

[4] *Ibid.*, 1879, Bd. xix. S. 563.

[5] Hammarsten, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. S. 333. It is unnecessary to add any ferment or nucleo-proteid to the solution to produce the result, but there is no doubt that nucleo-proteid may be present along with the fibrinogen. It was shown by Lilienfeld (*Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 89) that fibrinogen prepared by Hammarsten's method contains nuclein; from this he inferred that it is a nucleo-proteid, and not a globulin. But the amount of nuclein present is not sufficient

As just stated, fibrinogen is precipitated from plasma, and from its solutions in neutral salt solution, or dilute alkalies, by the addition of dilute acetic acid, even in slight excess. This precipitate has been termed "thrombosin" by Lilienfeld,[1] who regards it as due to a splitting of the fibrinogen under the influence of the acid into this substance and an albumose, but it has not been shown that it possesses any properties differing from fibrinogen.[2]

From what has been stated, it will be seen that it is improbable that the material which is obtained from plasma, under the name of fibrinogen, is a simple substance. It is probably either a mixture, or a loose combination, of at least three substances, namely—

1. *Fibrinogen proper*, coagulating at 56° C.
2. The globulin described by Hammarsten, and termed *fibrino-globulin*, coagulating at 65° C.
3. *A nucleo-proteid.*

The nucleo-proteid of plasma.—Beyond the fact of its presence, and that it appears to be one of the essential factors in the formation of fibrin, very little is known regarding the nucleo-proteid of blood plasma. It is doubtful if it exists in the plasma of circulating blood; it is thought by many that in this it is confined to the white corpuscles and blood platelets—a very little being also present in the red corpuscles—and that it is shed out by these as soon as the blood is drawn. The reasons for this belief are—

1. White blood corpuscles and similar cells (lymph cells, thymus cells, etc.) always contain a considerable amount of nucleo-proteid.
2. In plasma obtained by subsidence of the corpuscles there is most nucleo-proteid in the lower layers, which contain most leucocytes; and least in the upper, which contain very few.
3. Fluids which collect in the serous cavities of the body (pericardial fluid, hydrocele fluid, ascitic fluid) frequently contain no leucocytes. When this is the case they are also devoid of nucleo-proteid and of the property of spontaneous coagulability, although they contain fibrinogen.

The nucleo-proteid is precipitated from oxalate plasma, by allowing it to stand for twenty-four hours at 0° C. The addition of acetic acid in slight excess also throws it down, but not in a pure form, for fibrinogen is carried down along with it. Its solutions are coagulated at 65° C.; at a temperature of 60° C., in presence of free alkali, it is split into nuclein and a proteid. This is stated by Pekelharing to be a proteose,[3] but its proteose character is denied by Martin.[4] Halliburton and Brodie could also find no proteose in blood after the injection of nucleo-albumin.[5] In the presence of soluble salts of lime, it forms a

to justify this inference, and its presence is probably due to the fact that some or all of the nucleo-proteid present in the plasma is precipitated along with the fibrinogen, and clings to it in the subsequent processes of purification (Schäfer, *Proc. Physiol. Soc.*, *Journ. Physiol.*, Cambridge and London, 1895, p. xviii). See later, p. 172.

[1] *Loc. cit.*

[2] Schäfer, *Proc. Physiol. Soc.*, *Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. p. xx; Hammarsten, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxii. S. 384; Cramer, *ibid.*, 1897, Bd. xxiii. S. 74. According to Hammarsten, this coagulum, like that produced in a solution of the original fibrinogen, is not fibrin, but a fibrin-like combination of lime and fibrinogen. To me, however, it has often appeared difficult to distinguish from fibrin.

[3] Pekelharing, "Untersuch. ü. d. Fibrin-Ferment," Amsterdam, 1892.

[4] *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 375.

[5] *Ibid.*, 1894–5, vol. xvii. p. 159.

substance which possesses the property of converting fibrinogen into fibrin, and is, according to Pekelharing, a combination of the nucleo-proteid with lime, and identical with the fibrin ferment of A. Schmidt. The fibrin ferment is sometimes spoken of as "thrombin," and the nucleo-proteid material in the plasma from which it is produced is then termed "prothrombin."

Wooldridge[1] found that, on subjecting peptone plasma to cold, he obtained a finely granular deposit, which had the property of producing clotting in fibrinogenous fluids, which are not themselves spontaneously coagulable, and of accelerating the process of clotting in coagulable fluids. To the material thus obtained, and which he described as having, under the microscope, an appearance similar to masses of blood platelets, he gave the name "A-fibrinogen," because he found that on adding it to peptone plasma it produced fibrin, and that the amount of coagulation was more or less proportional to the amount of A-fibrinogen added. It is not fibrinogen as the term is ordinarily used, but is probably either a nucleo-proteid, or a mixture of nucleo-proteid with globulin. A similar deposit occurs, as already stated, in oxalate plasma, on standing in the cold. A precipitate containing the same substance is also produced by adding magnesium sulphate solution in considerable amount to blood, and in both plasma and serum of certain animals on acidulation with acetic acid, but in both cases it is liable to be mixed with serum globulin. It also occasionally occurs in serum, on standing, even without the application of cold. Halliburton has suggested that the deposit in peptone plasma may be a part of the proteoses, which were injected into the blood, for he found that solutions of albumose were liable to give a similar deposit on cooling by means of ice, but there is not enough proteose present in peptone plasma to account for such deposit, and the fact that it occurs under other conditions in plasma also negatives this supposition. These experiments of Wooldridge, and the behaviour of the body termed by him A-fibrinogen, will be again referred to in a subsequent section.

Fibrin.—Fibrin is the chief substance formed from fibrinogen in the coagulation of blood plasma, and it is also produced in the coagulation of lymph and other fibrinogen-containing fluids. It is usually got by whipping blood as it flows from the blood vessels with a bundle of wires or glass rods before it has had time to coagulate into a solid mass. The coagulum then forms upon the wires or rods, and can be washed free from adherent red corpuscles by putting it under a stream of water for a few hours. But to obtain pure fibrin it is necessary first to prepare fibrinogen from blood plasma by precipitation with NaCl (half-saturated), to purify this by re-solution and re-precipitation, and finally to cause the coagulation of the fibrinogen solution by fibrin ferment. The clot thus obtained, which must be thoroughly washed, is composed of nearly pure fibrin.

When obtained by whipping blood, fibrin is a white stringy substance when wet, drying to a glue-like mass. The threads of which it is composed, and which, as may be seen in a microscopic preparation of blood, interlace with one another and form a network of the finest possible filaments, entangling the blood corpuscles in its meshes, have a strong tendency to retract or shorten when formed; this is the reason why a clot shrinks and expresses serum from its interior. Fibrin is slowly soluble in 5 to 10 per cent. solutions of certain salts, such as sodium chloride, sodium sulphate, potassium nitrate, magnesium sulphate, and

[1] Wright, *Lancet*, London, 1892, vol. i. pp. 457, 515.

ammonium sulphate, and also in iodides and fluorides, and in solutions of urea.[1] It is also very slowly dissolved to some extent by normal salt solution; the solution is in all cases assisted by moderate warmth. Fibrin obtained from venous blood is slightly more soluble in salt solutions than that yielded by arterial blood. The proteid material which is found dissolved after solution of fibrin in the above salts is composed of two globulins,[2] having heat coagulation temperatures of 55° and 75° respectively. The latter, according to Halliburton, is reduced to 60°–65° in sodium chloride solutions, being 73°–75° in magnesium sulphate solutions only. Albumoses are also present in the fluid (Limbourg, Dastre). This solution of fibrin in neutral salts occurs in the entire absence of putrefactive decomposition (Green, Dastre). Fibrin swells in dilute acid (such as 0·2 per cent. HCl) into a clear jelly, which very slowly undergoes solution with the formation of acid albumin and proteoses. Stronger acids and, with the aid of heat, weak acids, effect the conversion more readily. The addition of pepsin to the acids employed greatly accelerates the conversion, the fibrin first splitting into two globulins, one coagulating at 56° and the other at 75°, and then becoming transformed into acid-albumin, proteoses, and peptones.[3] Trypsin in alkaline solutions has a similar action.[4]

Blood yields from ·2 to ·4 per cent. of its weight of dry fibrin. Hammarsten[5] gives the following as the elementary composition of fibrin:—

C . .	52·68	S . .	1·10
H . .	6·83	O . .	22·48
N . .	16·91		

It is, however, never free from ash, and the ash invariably contains lime,[6] but not more than other proteids,[7] nor does it contain more lime than the fibrinogen from which it is formed. Thus in one experiment Hammarsten found that a sample of fibrin, obtained by the action of ferment prepared from oxalated serum, upon fibrinogen prepared by precipitation from oxalated plasma by acetic acid, yielded exactly the same amount of lime as a sample of the fibrinogen itself, namely, 0·055 per cent. This fact completely disposes of the theories of coagulation which assume that fibrin is merely a combination of fibrinogen with lime, such as those of Freund, Arthus, Pekelharing, and Lilienfeld. Fibrin obtained by whipping blood leaves a considerable phosphorus-containing residue (nuclein) after subjection to peptic digestion; this is probably largely derived from the nucleo-proteids of the entangled leucocytes. But even fibrin obtained from solution of purified fibrinogen in dilute salt solution yields a certain amount of such residue.[8] It is possible that this may be an accidental impurity, but, on the other hand, it may be an integral constituent of the fibrin.

[1] Dastre, *Arch. de physiol. norm. et path.*, Paris, 1895, p. 408; *Compt. rend. Acad. d. sc.*, Paris, 1895, tome cxx. p. 589. See also on the solubility of fibrin in neutral salts, Holzmann, *Arch. f. Physiol.*, Leipzig, 1884, S. 210; and Arthus, "Coag. des liquides organiques," Paris, 1894, pp. 105 *et seq.*

[2] Green, *Journ. Physiol.*, Cambridge and London, 1887, vol. viii. p. 372.

[3] Hasebrock, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xi. S. 348.

[4] A. Herrmann, *ibid.*, Bd. xi. S. 508. The other literature on this subject will be found in Halliburton, "Text-Book of Physiol. and Path. Chemistry."

[5] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxii. S. 484.

[6] Frederikse, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xix. S. 143.

[7] Hammarsten, *ibid.*, Bd. xxii. S. 392.

[8] Schäfer, *Proc. Physiol. Soc., Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. p. xx.

Theories of Coagulation.

That the coagulation of the blood is due to the formation of an insoluble substance (fibrin) in the plasma, was proved by Hewson,[1] who showed that a coagulable plasma can be obtained by skimming, after allowing the corpuscles to subside, in blood the coagulation of which is delayed in any way, as by cold, by neutral salts, or by its retention within a living vein. The old theories which ascribed the coagulation to the cooling of the blood, to its coming to rest, to the running together of the corpuscles into rouleaux, were all effectually disproved by the same careful observer. Hewson also showed that fibrinogen ("coagulable lymph") is precipitable and removable from plasma by a temperature of a little over 50° C.[2] Many of Hewson's observations upon coagulation were forgotten, and the facts rediscovered by subsequent observers, but their accuracy was such that until comparatively modern times no addition of any permanent value to the knowledge of the subject was made. The most important of such additions (which was also overlooked for many years)[3] was the observation of Andrew Buchanan, that a substance could be extracted by water and solutions of salt from lymphatic glands, from blood clot (especially the buffy coat), and from various tissues, which had the property of producing the coagulation of serous fluids, not themselves spontaneously coagulable, such as hydrocele and pericardial fluid; such action being comparable to that of a ferment. But it is only quite recently that the active substance extracted by Buchanan has been examined, and found to belong to the class of bodies known as nucleo-proteids.

Schmidt's theory.—A theory of coagulation, which was long accepted, was that of Alexander Schmidt. Schmidt noticed that fluids which contained fibrinogen but were not spontaneously coagulable, such as pericardial or hydrocele fluid, coagulated on the addition of serum. He ascribed the fibrin formation which resulted to the action (fibrinoplastic action) of the globulin in the serum upon the fibrinogen of the pericardial fluid. Since, however, the same globulin is already present in abundance in pericardial and hydrocele fluid, it became clear that this explanation of the action of serum was insufficient. It was, however, shown by Schmidt that a substance is extracted by water from the alcohol precipitate of blood or serum, which possesses the property of causing coagulation in these fibrinogenous liquids, or of causing coagulation in plasma, the coagulation of which has been prevented by the addition of neutral salts. To this substance the name of fibrin ferment was applied, on account of its action resembling in general that of the unorganised ferments or enzymes. Thus it was found to have its activity accelerated by warmth, and destroyed by a high temperature (65° C.), and also to be capable of producing the coagulation of a relatively large amount of fibrinogen. It was still held by Schmidt that the globulin of serum takes an important share in the formation of fibrin.

Hammarsten's earlier researches.—Hammarsten showed that serum

[1] *Op. cit.*

[2] For the history of this see Schäfer, *Journ. Physiol.*, Cambridge and London, 1880, vol. iii. p. 185.

[3] Cf. Gamgee, "Physiol. Chemistry," 1880, vol. i., where will also be found an excellent account of the earlier history of the subject of blood coagulation. See also Arthus, "Coag. des liquides organiques," Paris, 1894, for a good epitome of the history of the subject up to that date.

globulin does not take part in forming fibrin. By precipitating fibrinogen by half-saturating plasma with sodium chloride, he obtained it free from serum globulin, and found that its solution in dilute salt solution was coagulated by the addition of Schmidt's extract—the so-called fibrin ferment—alone. Hammarsten proved that coagulation consists in a conversion of fibrinogen into fibrin; the change being accompanied by a splitting of the fibrinogen, and not by a combination of it with the serum globulin, as was supposed by Schmidt.

Influence of lime salts.—Theories of Freund and of Arthus and Pagès.—The more recent researches since these of Hammarsten have been in the direction of elucidating the true nature of the substances contained in Schmidt's extract. Green[1] found the extract to contain sulphate of lime, and that if lime were removed from plasma by dialysis its coagulability became lost, but was restored by the addition of sulphate of lime. Ringer and Sainsbury[2] showed that other salts of lime, such as calcium chloride, might replace the sulphate, and that the calcium might be replaced by barium and by strontium, although the salts of these metals are not so efficacious as the corresponding salts of calcium.

Freund[3] also drew special attention to the important part played by lime salts in promoting the formation of fibrin. He supposed the original cause of the deposition of fibrin in fibrinogenous liquids to be the formation of insoluble tribasic phosphate of lime, by the interaction of soluble phosphates (which he supposed to be shed out from the corpuscles whenever they come in contact with and adhere to foreign surfaces) with soluble lime salts contained in the plasma; the lime phosphate combining at the moment of formation with fibrinogen, and forming fibrin, and no other agency in the shape of a special ferment being necessary. This inference has not, however, been confirmed by subsequent observers. Freund supposed neutral salts, peptone, etc., to act in preventing coagulation, by keeping phosphate of lime in solution, and the walls of the blood vessels to act in preventing coagulation because the corpuscles do not adhere to them. Freund based his theory, partly upon the fact that if blood is drawn from an artery through a tube smeared with oil or vaseline into a vessel similarly prepared, the blood remains fluid for a long time, presumably because the adhesion of the corpuscles to the walls does not occur. Similar experiments with blood kept surrounded by paraffin or oil were performed by Haycraft with like result.[4]

Arthus and Pagès[5] mixed blood as it flowed from the vessels with a small quantity of a soluble oxalate[6] (0·07–0·1 parts per 100 of blood) sufficient to precipitate the lime salts dissolved in the plasma. They found that blood thus treated did not coagulate, however long it might be kept,[7] but that coagulability of its plasma is immediately restored on again adding a soluble lime salt, such as calcium chloride. They

[1] *Journ. Physiol.*, Cambridge and London, 1887, vol. viii. p. 354.
[2] *Ibid.*, 1890, vol. xi. p. 369.
[3] *Med. Jahrb.*, Wein, 1888, S. 259.
[4] *Journ. Anat. and Physiol.*, London, 1888, p. 172; Haycraft and Carlier, *ibid.*, p. 582.
[5] *Arch. de physiol. norm. et path.*, Paris, 1890, p. 739; Arthus, Thèse de Paris, 1892.
[6] Solutions of soap (0·5 parts per 100 of blood) or of soluble fluorides (0·2 parts per 100 of blood) act similarly to those of oxalate.
[7] Hammarsten makes a similar statement for horse's blood, but it is certainly not correct for all kinds of blood. Oxalate plasma, obtained from dog's or sheep's blood, does undergo coagulation on standing; coagulability is therefore not abolished by precipitation of the lime by oxalate, but merely deferred. We shall return to this point immediately.

inferred that the presence of a soluble salt of lime is necessary to the formation of fibrin, which, according to them, is produced by a combination, under the influence of fibrin ferment, of a part of the fibrinogen with lime, the remainder of the fibrinogen—which is assumed to split into two parts—forming a globulin coagulating at 64° C. (Hammarsten's fibrino-globulin).[1]

Whilst it would appear from these researches that soluble lime salts are necessary to the formation of fibrin,[2] it has been shown by Horne that the presence of a slight excess of these salts and also those of barium and strontium will hinder or, in great amount, entirely prevent its formation; their action being far more marked in this respect than that of other neutral salts, which require to be mixed in much greater amount with blood to prevent its coagulation.[3] The reason for this is probably to be found in the fact that fibrin is soluble to some extent in neutral salts of a certain strength (including salts of calcium, barium, and strontium).

Influence of nucleo-proteid.—Theory of Pekelharing.—Halliburton [4] and Pekelharing [5] both obtained from Schmidt's extract a body giving proteid reactions, and resembling in many particulars the globulins, to which class of proteids they at first regarded it as belonging.[6] They showed that the ferment action of Schmidt's extract is intimately dependent upon the presence of this substance, which could also, as Halliburton showed, be obtained from lymphatic glands. Halliburton termed it cell globulin; subsequently both observers recognised the fact that the substance in question was not a true globulin but a nucleo-proteid.[7] According to Pekelharing, it possesses the property of combining with lime, which it does not yield to distilled water by dialysis, nor is the combination broken up by soluble oxalates, although these, if present from the first, may prevent the original combination. The albumose in commercial peptone also prevents such combination, the albumose itself combining with the lime salts present;[8] if these are in excess, "peptone" does not prevent coagulation from taking place. The lime combination of nucleo-proteid is, according to Pekelharing, the body which has been known as fibrin ferment (thrombin). It can be formed not only from the nucleo-proteids contained in plasma or serum, but also from nucleo-proteids in the cells of the thymus, testicle, and other glands, by

[1] Arthus and Pagès found that strontium can replace lime in this reaction, but that barium and magnesium cannot. Ringer and Sainsbury have, however, shown that barium may take the place of lime in promoting coagulation, although it is less powerful (*Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 369). They also found that the salts of sodium and potassium act antagonistically to those of lime, barium, and strontium.

[2] A. Schmidt, even in his last communication upon the subject ("Weitere Beitr. z. Blutlehre," Wiesbaden, 1895), denied altogether that lime salts have any specific action or differed from other neutral salts, and considered that the addition of a soluble oxalate to blood acts either by preventing the formation of fibrin ferment or by hindering the action of ferment, if present, on fibrinogen. Cf., however, Arthus, *Arch. de physiol. norm. et path.*, Paris, 1896, and Hammarsten, *loc. cit.*

[3] *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. 356. Wright also noticed the fact that considerable excess of calcium added to oxalate blood prevents coagulation, *Journ. Path. and Bacteriol.*, Edin. and London, 1893, vol. i. p. 434.

[4] *Proc. Roy. Soc. London*, 1888, vol. xliv. p. 255.

[5] *Festschr. Rudolf Virchow*, Berlin, 1891, S. 435.

[6] Lilienfeld has recently repeated this error, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xx.

[7] Pekelharing, "Untersuch. ü. d. Fibrin-ferment," Amsterdam, 1892; Halliburton, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 312.

[8] Pekelharing. Cf., however, C. J. Martin, "Venom of Australian Black Snake," pp. 36–40, *Journ. and Proc. Roy. Soc. New South Wales*, Sydney, July 3, 1895.

digesting these with calcium chloride, the excess of calcium salt being afterwards dialysed off. Pekelharing supposes that the ferment action consists in the transference of lime from its nucleo-proteid combination to fibrinogen, the lime-compound of this being the insoluble fibrin,[1] and that if there is more lime salt in the solution the nucleo-proteid can recombine with lime, and thus become reconstituted as an agent for the conversion of fibrinogen into fibrin. As already pointed out (p. 166), however, it is not possible to accept this theory in view of the analyses of fibrin and fibrinogen given by Hammarsten. Pekelharing has himself shown that even in the entire absence of free lime salts, or in the presence of soluble oxalates, the transformation of fibrinogen into fibrin may be produced, provided that the ferment is present.[2] This has also been shown to be the case by A. Schmidt[3] and by myself,[4] and more recently in a series of carefully conducted experiments by Hammarsten. Hammarsten precipitated fibrinogen by oxalated solution of salt, and, after purifying it by repeated re-solution and re-precipitation, added to its solution a fibrin ferment obtained from oxalated serum, and obtained as the result a typical fibrin.[5]

Exception has been taken to the inference drawn by Pekelharing that Schmidt's ferment is a compound of nucleo-proteid and lime, on the ground that the ferment contained in Schmidt's extract differs from nucleo-proteids in the effect of alcohol upon its solubility in water, and in the fact that nucleo-proteids cause coagulation in intravascular plasma, which Schmidt's extract does not, whereas the latter causes coagulation in extravascular (salted) plasma, and nucleo-proteids do not.[6] The differences may, however, depend, in part at least, upon the relative amounts of nucleo-proteid and lime. Thus in Schmidt's extract the amount of nucleo-proteid is small and the amount of lime large; in extracts of thymus and the like the amount of nucleo-proteid is large and the amount of lime small. In part also they depend upon other circumstances, such as the influence of the magnesium sulphate of the salted plasma in antagonising the effect of lime.[7]

The origin of the nucleo-proteid of plasma and serum is probably the white corpuscles. It would appear that many of the latter disintegrate after removal of blood from the body. Gürber[8] found that in coagulated blood the number of white corpuscles was reduced to one-half, the difference being chiefly in the number of polynuclear cells. This disappearance has not, however, been found by all observers, and is not fully admitted. Nevertheless, without actually disintegrating, the white corpuscles may shed out or secrete nucleo-proteid into the plasma. This may occur normally in mere traces, but on with-

[1] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1895, No. 3.

[2] It would appear that a soluble oxalate does not throw down all the lime from a proteid-containing fluid, and that a trace of lime is still held in solution so that an oxalate plasma is not lime-free, as was supposed by Arthus and Pagès. This is well illustrated by an observation by Ringer upon the frog's heart, who finds that a normal saline solution, to which a little $CaCl_2$ has been added, will exhibit the physiological effect of lime, even after the addition to the fluid of a slight excess of a soluble oxalate. It may be inferred from this that a trace of lime may be held in solution even in a fluid destitute of proteid.

[3] "Weitere Beitr. z. Blutlehre," Wiesbaden, 1895.

[4] *Proc. Physiol. Soc.*, *Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. p. xviii.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xxii.

[6] Halliburton and Brodie, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvii. p. 143.

[7] Pekelharing, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1895, Bd. ix. S. 102. Halliburton in a recent paper (*Journ. Physiol.*, Cambridge and London, 1895, vol. xviii.) comes to a similar conclusion, namely, that Schmidt's fibrin ferment is a weak solution of nucleo-proteid. It produces Wooldridge's negative phase when intravenously injected.

[8] *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1892, No. 6.

drawal from the body in larger amount. It is noteworthy in this connection that certain forms of lymph, such as the aqueous humour, which contain no cells, contain also no nucleo-proteid, and are only coagulable on the addition of nucleo-proteid.

Theory of Lilienfeld.—Lilienfeld,[1] like Arthus and Pekelharing, considers that fibrin is formed by a combination of fibrinogen with lime, any soluble lime salt being equally effectual to produce the combination. Lilienfeld differs, however, from them in denying the necessity for the intervention of a ferment in the ordinary sense of the word. He considers that what the nucleo-proteid effects is not the combination of fibrinogen with lime, but a transformation or splitting of the fibrinogen into a substance which he terms "thrombosin," and a globulin; the thrombosin then combines with lime, if any be present, to form fibrin. The nucleo-proteid only acts, according to Lilienfeld, by reason of the acid qualities of the nucleic acid it contains. Any other weak acid, *e.g.* acetic, will answer equally well. Thus, if a solution of fibrinogen in NaCl (prepared according to Hammarsten's method) is precipitated by acetic acid, the precipitate (thrombosin), if dissolved in weak sodium carbonate, instantly forms a coagulum (fibrin), on the addition of calcium chloride. The formation of the thrombosin by the action of an acid upon fibrinogen is, according to Lilienfeld, a precursor to the production of fibrin, and is analogous to the change in caseinogen by the action of rennin, which will occur in the absence of lime salts, although the latter are necessary for the formation of the casein clot (see p. 135).

I have elsewhere shown [2] that this theory is untenable; for a solution of fibrinogen in dilute salt solution, prepared by Hammarsten's method, will, if sufficiently strong, coagulate, on the addition of calcium chloride, equally well with a solution of the acetic acid precipitate—the so-called thrombosin—although somewhat less rapidly.[3] The difference in rapidity depends, no doubt, upon the fact that sodium chloride in a certain amount retards the formation of the clot, or even may prevent it altogether. This, as Hammarsten has pointed out, is the reason why Lilienfeld obtained no coagulum on the addition of calcium chloride to his fibrinogen solution, although he got a coagulum with his so-called thrombosin solution, for the former was dissolved by aid of sodium chloride, and the latter by dilute alkali. As already stated,[4] Hammarsten holds that in neither case is the coagulum produced a true fibrin, but in both cases it is a fibrin-like combination of fibrinogen with lime. The influence of nucleo-proteid is, however, not eliminated,[5] for, as has been already insisted on, fibrinogen prepared by Hammarsten's method always contains some nucleo-proteid. This is clear both from my own experiments and from the analyses of Lilienfeld, who indeed—but as it would appear without sufficient cause—supposes fibrinogen itself to be a nucleo-proteid. The amount of nuclein or phosphorus which can be obtained from it certainly does not warrant the assumption; nevertheless there is always a distinct

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx.

[2] *Proc. Physiol. Soc., Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. p. xviii.

[3] Cf. Cramer, *Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. xxiii. S. 74, who has fully confirmed the conclusion that the so-called "thrombosin" is merely fibrinogen.

[4] See note 5, p. 165.

[5] Cf. Wistinghausen, Diss., Dorpat, 1894.

residue after gastric digestion of its solution, showing that it at least, as above stated, contains some nucleo-proteid. Probably this is an accidental contamination.

Production of intravascular coagulation of blood and of uncoagulable blood.—It was discovered by Edelberg[1] that intravenous injection of Schmidt's fibrin ferment may produce thrombosis in the venæ cavæ, the right side of the heart, and the pulmonary arteries. Foà and Pellacani[2] showed that the same will occur with extracts of various organs. The same fact was independently noticed by Wooldridge,[3] who found that a substance or substances obtainable from saline extract of lymphatic glands, thymus, testicle, and other glandular organs, tend to produce, when injected rapidly in sufficient amount into the veins of animals, instant coagulation of the blood whilst still within the blood vessels. On the other hand, if injected more slowly, or in insufficient amount to produce intravascular coagulation, the coagulability of the blood *in vitro* becomes abolished; this condition was termed by Wooldridge the "negative phase." When the negative phase is once obtained, a very large dose of the material fails to produce intravascular clotting.[4] Wooldridge gave the name "tissue fibrinogens" to the substances thus extracted, and more extended knowledge has led to the general recognition of the fact that they belong to the class of nucleo-albumins or nucleo-proteids. The coagulation when it occurs is found, first, in the portal venous system; then in the general venous system, and pulmonary arteries and in the right side of the heart; and finally, when the effect is most pronounced, in the general arterial system; but rarely in the pulmonary veins. Its occurrence is assisted by an excess of CO_2 in the blood.[5] Albino rabbits and the Norway hare in its albino condition are immune to these effects (Pickering).

It has been supposed by Lilienfeld[6] that this action of the nucleo-proteid in causing coagulation is due to the nuclein or nucleic acid which it contains, and that, when the negative phase is obtained, this result is due to the action of the proteid part of the molecule of nucleo-proteid in preventing coagulation. This hypothesis is rendered improbable by the observations of Halliburton and Pickering,[7] who found that intravascular coagulation can be readily obtained in rabbits by intravenous injection of artificial colloids (containing no nucleic acid).[8] These colloids likewise yield the negative phase (retardation of coagulation), if injected in quantity insufficient to produce coagulation; and, as with solution of nucleo-proteids, they are without action upon albino rabbits. Nevertheless, like solutions of nucleo-proteid, they hasten the coagulation of the blood of other animals, if mixed with it *in vitro*. These observers also found that the retarding influence of

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1880, Bd. xii. S. 283.
[2] *Riv. clin. di. Bologna*, 1880, p. 241.
[3] *Proc. Roy. Soc. London*, 1886. See also "Die Gerinnung des Blutes," Leipzig, 1891.
[4] Wooldridge, *Arch. f. Physiol.*, Leipzig, 1888.
[5] Wright, *Journ. Physiol.*, Cambridge and London, vol. xii. [6] *Loc cit.*
[7] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. pp. 54 and 285; Pickering, *Proc. Roy. Soc. London*, 1896, vol. lx. p. 337.
[8] The artificial colloids investigated were prepared by Grimaux's methods (*Compt. rend. Soc. de biol.*, Paris, 1881, tome xciii. p. 771; 1884, xcviii. pp. 105, 1434, and 1578). Their chemical properties and mode of preparation have already been described by Professor Halliburton in a previous article (p. 36). It is possible that they may act, not directly, but by setting free nucleo-proteid from the white corpuscles. Their solutions do not, however, cause disintegration either of the red or white corpuscles, nor any apparent change in the epithelium of the vessels.

intravascular injections of soap, peptone, and potassium oxalate on the coagulation of blood is antagonised by previous intravascular injection of the colloid. The action of the colloids in promoting coagulation is assisted, like that of nucleo-proteid, by accumulation of CO_2 in the blood. The same effects are produced by snake venom,[1] which contains no nucleo-proteid, and the active principles of which consist of albumoses. This produces, although in far more minute doses, effects which are in every way comparable with those produced by Wooldridge's "tissue fibrinogen." In doses of 0·00001 to 0·00002 grm. per kilog. body weight, the venom of the Australian black snake (*Pseudechis porphyriaca*) causes the blood, after a brief interval of increased coagulability (positive phase), to lose its tendency to clot (negative phase), and much larger doses of the poison will now not restore its coagulability. On the other hand, moderate and large doses (more than 0·0001 grm. per kilog.) produce instantaneous clotting within the vessels. But any blood which has not undergone the intravascular coagulation is found to be incoagulable *in vitro*, and in this point also there is an exact resemblance to the phenomena produced by nucleo-proteids and by artificial colloids.

Solutions of certain other chemical substances, such as ether, tannic acid, arsenic,[2] glycerin, toluylenediamin,[3] are also found when injected into the circulation to produce thrombosis. But, to produce the effect, these all require doses large enough to cause disintegration of the blood corpuscles, thereby setting free the nucleo-proteids which the corpuscles contain, so that their action is probably a secondary one. It is possible that snake venom may also operate in this way,[4] since it does produce to a certain extent such disintegration, but the rapidity of the production of the intravascular clotting, and the small amount of such disintegration which normally occurs, render such an explanation improbable.

Peptone plasma.—Researches of Wooldridge.—Other substances, such as commercial peptone, the action of which is due to the albumoses which it contains, and leech extract, produce a diminution or loss of coagulability when injected into the blood vessels, without, in any dose, tending to cause intravascular coagulation. The incoagulable blood or plasma obtained by their employment resembles very closely that obtained in the negative phase, produced by Wooldridge's tissue fibrinogen, by colloids and by snake venom. Peptone plasma can be made to coagulate by

1. Addition of lymph cells.
2. Addition of nucleo-proteids.
3. Addition of calcium chloride.
4. Dilution with water, or 0·5 per cent. salt solution.
5. A stream of CO_2.
6. Neutralisation with acetic acid.

But if an excess of the reagents employed to prevent coagulation (or to produce the negative phase), whether peptones or slowly

[1] C. J. Martin, *Journ. Physiol.*, Cambridge and London, 1893, vol. xv. p. 380; and *Journ. and Proc. Roy. Soc. New South Wales*, Sydney, July 3, 1895. These papers contain full references to the previous literature of the subject.

[2] The administration of arsenic and phosphorus by the mouth *diminishes* the coagulability of the blood (cf. Gley and Pachon, *Arch. de physiol. norm. et path.*, Paris, 1896, p. 716).

[3] Silbermann, *Virchow's Archiv*, 1889, Bd. cxvii. S. 288.

[4] C. J. Martin, *op. cit.*, *Journ. and Proc. Roy. Soc. New South Wales*, Sydney, pp. 45–47 of reprint.

administered nucleo-proteids or snake venom be employed, then these additions do not produce coagulation.[1] In view of the fact that calcium chloride will not, under these circumstances, produce coagulation, the hypothesis of Freund and Pekelharing, that "peptones" deprive blood of its coagulability by combining with its calcium salts, loses probability.

Peptone injected intravenously rapidly disappears from the blood.[2] The action of peptone differs from that of leech extract, in that a second dose, given soon after the action of the first dose has passed off, fails to produce an effect on coagulability. Moreover, the blood of a "peptonised" dog confers immunity from the action of peptone, if injected intravenously into a second animal.[3]

The properties of peptone plasma, and the effects of leucocytes and their saline extracts upón the coagulability of blood, were carefully studied by Wooldridge. This observer found, as already stated, that if peptone plasma be kept for a time at 0° a precipitate forms, which takes the form, under the microscope, of minute discoid particles, almost exactly similar to blood platelets. The substance thus precipitated was termed "A-fibrinogen" by Wooldridge, while he named the substance precipitable by half-saturation with NaCl "B-fibrinogen"; this is the same thing as fibrinogen as ordinarily understood. After the removal of the A-fibrinogen, the coagulability of peptone plasma by CO_2 and other conditions is greatly diminished or altogether lost, but is restored on dissolving the A-fibrinogen again with the aid of warmth.

Wooldridge's A-fibrinogen is also obtainable, as Wright has shown, by cooling oxalate plasma, and it is probably composed mainly, if not entirely, of nucleo-proteid. It has been shown by Hammarsten that if by prolonged cooling and filtration it is removed as much as possible from oxalated plasma, the plasma will not coagulate on the addition of sufficient lime salts to more than balance the excess of oxalic acid, but that, if the precipitate be collected and treated with lime salts, it furnishes, on subsequently removing the lime by oxalate, a powerful *thrombin* or fibrin ferment. Hammarsten accordingly terms the substance in question, which is precipitated by cold from plasma, *prothrombin*, and considers that it can only be converted into thrombin by the action upon it of lime salts. Pekelharing regards the precipitate in question as composed of nucleo-proteid, and considers that the lime acts by combining with it to form fibrin ferment.

The addition of lymph cells (washed with 0·6 per cent. NaCl solution) to peptone plasma causes its coagulation outside the body, and also accelerates the coagulation of ordinary blood *in vitro*, whereas the intravenous injection of salt solution holding these washed lymph cells in suspension produces an incoagulable condition (negative phase) of blood, which does not then coagulate, even on withdrawal. But on addition of some of this fluid, holding cells in suspension, to such blood after withdrawal, coagulation is rapidly produced. The loss of coagulability of peptone plasma is not due, as was supposed by A. Schmidt, to the disappearance and disintegration of leucocytes, for, as Wooldridge showed, the addition

[1] C. J. Martin, *op. cit.*, pp. 35–40. According to Dastre and Floresco, the chief cause of the lack of coagulation in peptone plasma is its high alkalinity (*Arch. de physiol. norm. et path.*, Paris, 1897, p. 216).

[2] Schmidt-Mulheim, *loc. cit.*

[3] Contejean, *Arch. de physiol. norm. et path.*, Paris, 1895.

of leucocytes to the circulating blood does not increase its coagulability, but the contrary; and, moreover, peptone plasma contains many leucocytes.[1] These several facts were explained by Wooldridge by the supposition that coagulation is produced or prevented in the absence of leucocytes by the action of one substance in the plasma upon another, or in the presence of leucocytes by the action of a substance within the plasma upon these cells, or material yielded by them; the kind of interaction being different under different circumstances, and producing, respectively, the phase of incoagulability or coagulation (negative or positive phase) according to such circumstances. All such substances, which by their interaction tended to produce fibrin, were termed by Wooldridge "fibrinogens"; but the progress of research has since rendered it probable that Wooldridge's "A-fibrinogen" obtained from plasma, his "serum fibrinogen" obtained from dog's serum, and the "tissue fibrinogens," which he obtained from various organs, all owe their action to the nucleo-proteid which they contain. Translating, then, the phraseology employed by Wooldridge, the alterations in blood plasma, which come under the various conditions above noticed, are due to the interaction of nucleo-proteids and fibrinogen. And it would appear that, when in the interaction the nucleo-proteids are present in relatively small amount, the negative phase is the result; when in large amount, the positive phase. Also that, when added to the circulating blood, leucocytes yield but little of their nucleo-proteid to plasma, and hence a negative phase is the result; but, on the other hand, when added to plasma *in vitro*, a larger amount is yielded, and coagulation results. A remarkable observation, and one very difficult to explain, is the fact that, if the negative phase is once established by the intravascular injection of a small amount of nucleo-proteid, artificial colloid, or snake venom, a large excess of the same will then not only fail to produce the positive phase, but will even strengthen the negative phase. It is, therefore, only the initial change which is influenced by the relative amounts of interacting material; and, when once this change is established, it does not again become modified.

Wright's experiments.—Wooldridge further found that under some circumstances the amount of fibrin produced was dependent upon the amount of tissue fibrinogen or A-fibrinogen (nucleo-proteid) added to plasma. He therefore came to the conclusion, since the extent of action was not in all cases independent of the amount of these substances, that the action could not be looked upon as that of a ferment, although under some circumstances the extent of action did appear to be independent of the amount of these substances. Wooldridge offered no explanation of the different effects obtained with large and small doses respectively, his work upon the subject having been cut short by his untimely death. It has, however, been continued on the same lines by Wright,[2] who, whilst confirming most of Wooldridge's observations, has added materially to our knowledge of the conditions under which "tissue fibrinogen" or nucleo-proteids produce the negative and positive phase of coagulability. Wright states that the extracts of glands containing

[1] Wright found, however, that the number of leucocytes in peptone blood was extremely reduced, much more so than is the case in oxalate or magnesium-sulphate blood, and that it contains a correspondingly larger amount of nucleo-albumins, *Proc. Roy. Soc. London*, 10th Feb. 1893.

[2] *Proc. Roy. Irish Acad.*, Dublin, 1891, 3rd series, vol. ii. p. 117.

these substances readily yield, under the influence of certain reagents and conditions, a body or bodies giving albumose reactions; and he finds that such a body is also present in the blood after their injection, and rapidly appears in the urine.[1] Wright considers it probable, therefore, that the contrary effects of large and rapid, or small and gradual, administration of these extracts is due in the one case to the immediate action of the nucleo-proteids in effecting the conversion of the fibrinogen into fibrin before there has been time for the formation of albumose; and in the other case, where there has been time for such formation, to the action of the albumose thus formed in preventing coagulation (as in the case of directly injecting albumose into the blood vessels). If any albumose is formed, the action of this would, by delaying coagulation, give time for the formation of more, when a second dose of nucleo-proteids is injected. Hence, a dose of nucleo-proteid, which would, if administered rapidly, produce instantaneous coagulation throughout the vascular system, may, if administered gradually, tend altogether to prevent coagulation. But, as Halliburton points out, the explanation of the action of "peptone" in producing a negative form of coagulation may be that it liberates small quantities of nucleo-proteid, rather than that it removes calcium; and if this is so, the explanation offered by Wright (and Pekelharing) of the action of nucleo-proteids falls to the ground. Moreover, it cannot be accepted as proven that a "peptone" moiety is split off from nucleo-proteid. "Peptone" (*i.e.* "albumose")-blood is characterised by extreme diminution of the amount of CO_2 which it contains,[2] and by diminished alkalinity,[3] and the reason for the uncoagulability of such blood is apparently connected with its deficiency in CO_2 tension,[4] since it coagulates on passing a stream of CO_2 throughout it. For the occurrence of intravascular coagulation, after injection of nucleo-proteid and similarly acting substances, is largely influenced by the amount of CO_2 in the blood, and it is due to its richness in CO_2 that the blood coagulates under these circumstances, first in the systematic veins, and of these most readily in the portal venous system.[5]

From what has been before said as to the influence of lime, it will be understood that the lime-salts of the plasma play an essential part in the interaction between the nucleo-proteid and the fibrinogen. This participation of lime in the reaction had not yet been recognised when Wooldridge's researches were made, but is freely admitted by Wright, whose views upon the subject of the combined action of nucleo-proteid and lime in producing coagulation seem to be in close agreement with those of Pekelharing (see p. 171). It is, however, still by no means clear why in "peptone" plasma, where all the necessary factors for the formation of fibrin are present, coagulation, nevertheless, does not occur,

[1] This has been also shown independently by Pekelharing ("Untersuch. u. d. Fibrinferment," Amsterdam, 1892), who offers a similar explanation of the phenomenon of negative and positive coagulation. Halliburton and Pickering, on the other hand, consider that, in the case of colloids, the negative phase cannot be regarded as a subsidiary phenomenon, due to disintegration of the material intravenously injected, but is rather a result characteristic of the action of small doses, and is comparable to the inhibitory action of small doses of certain drugs, which act contrary to the action of larger doses (such as the physiological immunity produced by small doses of alexines).

[2] Lahousse, *Arch. f. Physiol.*, Leipzig, 1889, S. 77.

[3] Salvioli, *Arch. ital. de biol.*, Turin, 1892, vol. xvii. p. 155.

[4] Wright, *loc. cit.*, and *Journ. Path. and Bacteriol.*, Edin. and London, 1893, vol. i. p. 434.

[5] Wright, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii.

although it speedily occurs on further addition of lime or on passing CO_2. Wright[1] assumes that the nucleo-proteid acting as a weak acid has ousted CO_2 from the bases of the plasma, and that the action of CO_2 is to set the nucleo-proteid free again. But this would not account for the effect of addition of calcium chloride. It may, on the other hand, be that the lime which is present in the plasma is unable to act upon the nucleo-proteid also present, owing to the former having entered into some combination from which it is set free by CO_2. It must be admitted that the subject is still, in spite of much research, enveloped largely in obscurity.

Influence of the liver and lungs upon blood coagulability.—It was shown by Pawlow[2] that if blood be allowed to circulate through the heart and lungs only, and be cut off from the rest of the body, it gradually loses its coagulability, and the same observation was made independently by Newell Martin.[3] Bohr[4] obtained a similar result, on preventing the blood from reaching the portal circulation by occluding the thoracic aorta. The blood lost its coagulability in a quarter of an hour, nor was it restored for twenty-four hours after readmission to the abdominal viscera. This was in the dog. In a rabbit, ligature of the cœliac axis and mesenteric arteries produced a similar but rather less pronounced effect. Delezenne has shown that artificial perfusion of "peptone-blood" through the liver restores its coagulability, but that other organs do not produce the same result.[5]

Gley and Pachon[6] find, in confirmation of Contejean,[7] that every cause which diminishes or suppresses the functional activity of the liver diminishes or suspends the anti-coagulating action of "peptone." They thus explain the experiments of Contejean, who noticed that after extirpation of the cœliac ganglia the action of "peptone" is not obtained.[8]

Hédon and Delezenne[9] also found that after the establishment of an Eck's fistula (communication between portal vein and vena cava) in the dog, and the subsequent removal of the liver, injection of "peptone," although it produces a great fall of blood pressure, no longer removes the coagulability of the blood. These experiments appear to show that the liver has a special function in connection with the maintenance of the coagulability of the blood, and that in passing through the lungs an effect of an opposite character is produced, but in what way exactly these organs exert their influence has not as yet been ascertained.

Blood or plasma can be temporarily made uncoagulable in the living vessels by removing the fibrin. Dastre found that, if a large quantity of blood be drawn from an animal, and this be whipped and filtered and returned to the blood vessels, and the process repeated two or three times, all the fibrin can be temporarily removed; and it is only gradually that the blood resumes its coagulability, which is not completely restored until the lapse of some hours.

Conclusions regarding the causes of coagulation.—At least three factors appear necessary to effect the formation of fibrin, namely,

[1] *Journ. Path. and Bacteriol.*, Edin. and London, 1893, vol. i. p. 434.
[2] *Arch. f. Physiol.*, Leipzig, 1887, S. 458.
[3] Quoted by Gad, *Verhandl. d. Berl. phys. Gesellsch., Arch. f. Physiol.*, Leipzig, 1887, S. 584.
[4] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1888, S. 261.
[5] *Compt. rend. Acad. d. sc.*, Paris, 11 Mai, 1896, p. 1072.
[6] *Arch. de physiol. norm. et path.*, Paris, 1895, p. 711.
[7] *Ibid.* p. 245.
[8] *Ibid.*, 1896, p. 159.
[9] *Compt. rend. Soc. de. biol.*, Paris, 1896, p. 633.

fibrinogen, nucleo-proteid (prothrombin), and lime, and it would appear probable from Pekelharing's researches that the two latter act in combination, and in fact represent the body which was termed by Schmidt the fibrin ferment (thrombin). The reason why in the healthy living vessels the blood does not coagulate, is, in all probability, that the nucleo-proteid and lime have not entered into the necessary combination or interaction which enables them to act as a ferment upon the fibrinogen.[1]

Of the three factors above mentioned, it is certain that fibrinogen and lime are both present in the plasma of circulating blood, and the problem therefore resolves itself into the question whether nucleo-proteid is present or not, or whether, if present, it is in a different condition from that necessary to promote fibrin formation. We may consider the latter question first, and in doing so it will be convenient to assume, as the experiments of Pekelharing seem to have proved, that the fibrin ferment of Schmidt is a product of the interaction of nucleo-proteid with lime. This conclusion of Pekelharing's has, in fact, been confirmed by the researches of Hammarsten, who has shown that the nucleo-proteid (or prothrombin) which is obtainable from plasma is inactive as a ferment, except in the presence of or after it has been exposed to the action of soluble lime salts. It is not, however, equally clear that the fibrin ferment is a *compound* of the prothrombin with lime, as Pekelharing supposed it to be.

Is fibrin ferment present in the plasma of circulating blood? As is well known, Schmidt's fibrin ferment is ordinarily obtained from clotted blood or from serum, and the ferment-like substance used by A. Buchanan was also obtained by him from blood clot, and especially from buffy coat, *i.e.* the portion containing most white corpuscles. Schmidt found that if blood were drawn from the vessels direct into alcohol, no ferment could be obtained from it.[2] He came, therefore, to the conclusion that the blood does not coagulate in the living vessels owing to the absence of fibrin ferment, and that this is only formed or set free when the blood is drawn. Since fibrin ferment could be obtained in greatest abundance from the layer of the clot where leucocytes are most abundant, and from other tissues and organs rich in similar cells, it appeared probable that it is derived in drawn blood from the white corpuscles, and, as Schmidt believed, from their disintegration. Such disintegration of leucocytes was in fact described by Schmidt in drawn blood, but the observation has not been generally confirmed. It is not, however, necessary to suppose disintegration of the corpuscles, for they may shed out the ferment without actually undergoing disintegration. Now, conditions which render the blood incoagulable, such as injections of "peptones," of snake venom, and nucleo-proteids in small amount, greatly diminish the number of leucocytes in the blood. This they do, however, not by causing the solution and disintegration of the corpuscles,

[1] Hammarsten, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxii. We may dismiss the hypothesis of Astley Cooper (Thackrah, "An Essay on the Cause of the Coagulation of the Blood," *Med.-Chir. Rev.*, London, 1807, p. 191), which was revived by Brücke, (*Brit. and For. Med.-Chir. Rev.*, London, 1857, and *Virchow's Archiv*, 1857, Bd. xii.), that the living vascular walls exercise by their presence a restraining action upon coagulation, as having been sufficiently disproved by Lister ("On the Coagulation of the Blood," *Proc. Roy. Soc. London*, 1863).

[2] Subsequent researches conducted in his laboratory have shown that a very small amount is obtainable even under these conditions.

as supposed by Löwit[1] and by Wright,[2] but by causing their accumulation within the tissues (? capillary blood vessels). For a very short time after their almost complete disappearance from the blood they begin gradually to reappear, and in one experiment C. J. Martin found that the full number had reappeared within as short a time as fifteen minutes.[3] Moreover, if such disintegration really took place one would expect the coagulability of the blood to be visibly increased, from the setting free of their nucleo-proteids, whereas it is actually diminished or abolished. Nevertheless, those substances, such as snake venom, nucleo-proteids, and colloids, which in larger doses produce intravascular coagulation, may in part act by causing disintegration, if not of leucocytes at least of red corpuscles which also contain nucleo-proteids. That this occurs to some extent is shown by the fact that the serum is usually tinged by hæmoglobin. And even without actual disintegration the permeability of the corpuscles may become altered, and nucleo-proteid shed out.

But there is another tissue upon which the reagent in question may act, namely, the epithelial cells of the blood vessels. These are in all probability composed of living protoplasm, and the reagents may either cause them to shed out nucleo-proteid and so produce fibrin ferment, or, by deleteriously affecting them, may cause them to react upon the leucocytes which are passing along in contact with their inner surface, and effect a discharge of nucleo-proteid from these cells. That snake venom affects the blood vessels deleteriously is shown by the capillary hæmorrhages which are so frequently seen after poisoning by it, and by the rapid effect it produces on the blood circulating in the mesentery, if a little be applied to the surface of that membrane.[4] The same does not, however, obtain with artificial colloids, nor with nucleo-proteids; although, with partial blocking of the portal vein, after injection of a small dose, capillary hæmorrhages have been found to occur in the liver.[5]

The evidence which we have had before us points to the following conclusions regarding coagulation:—

1. That the coagulation of blood, *i.e.* the transformation of fibrinogen into fibrin, requires for its consummation the interaction of a nucleo-proteid (prothrombin) and soluble lime salts, and the consequent production of a ferment (thrombin).

2. That either nucleo-proteid is not present in appreciable amount in the plasma of circulating blood, or that the interaction in question is prevented from occurring within the blood vessels by some means at present not understood.

3. That the nucleo-proteid (prothrombin) appears and the interaction occurs, as soon as the blood is drawn and is allowed to come into contact with a foreign surface, the source of the nucleo-proteid being in all probability mainly the leucocytes (and blood-platelets ?).

4. That, under certain circumstances and conditions, either the nucleo-proteid does not appear in the plasma of drawn blood, or it appears, but the interaction between it and the lime salts is prevented or delayed.

[1] "Stud. z. Physiol. u. Path. d. Blutes," Jena, 1892.
[2] *Proc. Roy. Soc. London*, 1893, vol. lii.
[3] *Loc. cit.*
[4] Weir Mitchell and Reichert, "Researches upon the Venoms of Poisonous Serpents," *Smithson. Contrib. Knowl.*, Washington, vol. xxvi.
[5] Wooldridge, *Trans. Path. Soc. London*, 1888, p. 421.

5. That the nucleo-proteid (prothrombin) appears in the plasma of *circulating blood* under certain conditions, being in all probability shed out from the white corpuscles and blood platelets, or in some cases even from the red corpuscles; and that when shed out under these conditions from the corpuscles, or when artificially injected into the vessels, it tends at once to interact with the lime salts of the plasma and to form fibrin ferment (thrombin), intravascular coagulation being the result.

6. That, under other conditions, either the shedding out of nucleo-proteid from the corpuscles, or its interaction with the lime salts of the plasma, may be altogether prevented and the blood rendered incoagulable, unless nucleo-proteid be artificially added, or unless a modification of the conditions is introduced which will permit of the interaction of the nucleo-proteid with lime to form ferment.

7. That the nucleo-proteid (prothrombin) is incompetent, in the entire absence of lime salts, to promote the transformation of fibrinogen into fibrin; but, as a result of its interaction with lime salts, it becomes transformed into a ferment (thrombin), which, under suitable conditions of temperature and the like, produces fibrin.

8. That either the place of nucleo-proteid in coagulation may be taken by certain albumoses, such as those found in snake venom, and by certain artificial colloidal substances, such as those prepared by Grimaux, or that such substances may act by setting free nucleo-proteid from the leucocytes and other elements in the blood, or from the cells of the blood vessels, and thus indirectly promote coagulation.

If the former supposition is the correct one, in all probability these three substances (nucleo-proteid, snake venom, albumose, and colloid of Grimaux) contain the same active molecular group.[1]

Lymph, Chyle, Serous Fluids, Cerebro-Spinal Fluid, Synovia.

Lymph, which is obtainable from the lymphatic vessels of the limbs, from the thoracic duct, and from the lacteals in the intervals of absorption of digestive products, or from the serous cavities—although only occurring normally in sufficient amount for purposes of analysis and experiment in the pericardial cavity—resembles generally in the character of its constituents, but not in their relative amount, the plasma of the blood. Nor are the proportions of its constituents so constant as are those of blood plasma, for there is reason to believe that the lymph from different organs presents very considerable differences in their relative amounts.

Lymph has generally been obtained for analysis from accidental lymphatic fistulæ in man, from experimental fistulæ in large animals, such as the horse, or from the thoracic duct of fasting animals (dog). The amount flowing along the thoracic duct is about 64 c.c. per kilog. body weight per diem.[2]

[1] Halliburton and Pickering, *op. cit.*

[2] R. Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlix. S. 216; Nöel Paton, *Journ. Physiol.*, Cambridge and London, 1890, vol. xl. p. 109.

The following analysis is given by J. Munk and Rosenstein from a case of fistula of the thoracic duct in man:[1]—

In 100 parts lymph—	
Total solids	3·7–5·5
Proteids	3·4–4·1
Substances soluble in ether	0·046–0·13
Sugar	0·1
Salts	0·8–0·9
Nacl	0·55–0·58
Na_2CO_3	0·24

Hensen and Dänhardt[2] found the following inorganic constituents:—

In 100 parts lymph—	
NaCl	0·614
Na_2O	0·057
K_2O	0·049
CaO	0·013
MgO, Fe_2O_3	Traces
CO_2	0·0815
SO_3, P_2O_5	0·033

They obtained only 0·1 per cent. of fibrin (as compared with 0·4 per cent. in blood plasma). This is perhaps the reason why the intravenous injection of peptone in small amount or at a slow rate may, as noticed by L. E. Shore,[3] prevent the clotting of the lymph but not that of the blood. The experiments of Spiro and Ellinger[4] seem, however, to indicate that, under the influence of peptone, an anti-coagulating substance is formed in lymph, and from this passes into the blood. The other proteids are also present in much less amount, but the relative proportion of albumin to globulin is almost exactly maintained. As already stated (p. 162), the present proteid quotient is fairly constant in the same individual, both for blood serum, lymph serum, and serous effusions.[5] Lymph generally contains more urea than does the blood of the same individual. Thus in a dog Wurtz found—

In the blood, 0·009 parts per cent. of urea.
In the lymph, 0·016 „ „ „

The amount of sugar in lymph is about the same as in blood plasma, although, if dextrose be injected into the blood vessels, it soon appears in greater proportion in the lymph than in the blood.[6] Lymph contains a distinct amount of glycogen, but this substance is wholly contained in the corpuscles, and none exists in the plasma.[7]

The aqueous humour is a form of lymph, and contains the same proteid substances as lymph, namely, fibrinogen, serum globulin, serum albumin, and similar extractives and salts.[8] It contains no corpuscles,

[1] *Arch. f. Physiol.*, Leipzig, 1890, S. 376; *Virchow's Archiv*, 1891, Bd. cxxiii. S. 230 (contains a historical account of other cases).
[2] *Virchow's Archiv*, 1866, Bd. xxxvii.
[3] *Journ. Physiol.*, Cambridge and London, 1890, vol. xl. p. 561.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. xxiii. S. 121.
[5] Salvioli, *Arch. f. Physiol.*, Leipzig, 1881, S. 269; Hofmann, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1882, Bd. xvi. S. 135.
[6] The reasons for this will be considered in the article on "Lymph Production."
[7] Dastre, *Compt. rend. Acad. d. sc.*, Paris, 1895, tome cxx.
[8] Halliburton and Friend, *Rep. Brit. Ass. Adv. Sc.*, London, 1889.

and does not clot spontaneously, but only on addition of fibrin ferment, such as is contained in serum. Traces of urea are present but no sugar, although a slight reduction of Fehling's solution is sometimes obtainable. This is probably due to paralactic acid.[1]

The following analysis of aqueous humour is by Lohmeyer:[2]—

In 1000 parts—

Water	986·87
Proteids	1·22
Extractives	4·21
NaCl	6·89
Other salts	0·81

Pericardial fluid is a form of lymph which is found in small quantity within the sac of the pericardium. Peritoneal and pleural fluids, and the fluid of the tunica vaginalis, are not normally present in sufficient quantity to be collected and analysed. Pericardial fluid contains rather less proteid than ordinary lymph (2·28–2·55 per cent.).[3]

Pericardial fluid, as obtained from the horse or ox, is a yellowish fluid, resembling serum in appearance and in its general composition, but it contains fibrinogen.[4] It usually has no leucocytes, nor is it spontaneously coagulable, but it coagulates on the addition of ferment or of nucleo-proteid.

Chyle has nearly the same composition as lymph, but it contains more solid matter, the increase being chiefly in fats, but also in proteids. The following table from Hoppe-Seyler gives its general composition in the dog and a comparison with the serum of the same animal.

	Chyle of Dog.	Serum of same Dog.
Water	90·67	93·60
Fibrin	0·11	...
Albumin and globulin	2·10	4·52
Fat, lecithin, cholesterin	6·48	0·68
Other organic substances	0·23	0·29
Salts	0·79	0·87

The ether extract of chyle was found by Hoppe-Seyler to contain, per cent.:—

Cholesterin	14·09
Lecithin	8·84
Fats	77·07

There is also, according to Hoppe-Seyler, a small amount of soap in chyle. The amount of urea and of sugar is about the same as in lymph.

The cerebro-spinal fluid, although resembling lymph in its appearance, and probably in being formed by transudation from the blood vessels, differs from lymph chemically in certain important details. Although cerebro-spinal fluid is not obtainable normally in sufficient amount for analysis, the fluid of a meningocele appears to be nothing but an accumulation of the normal fluid, and has been frequently analysed, and the fluid of hydrocephalus has also been used for this purpose. Cerebro-spinal fluid as thus obtained is a clear, colourless

[1] Kuhn, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xi. S. 200; Grünhagen, *ibid.*, S. 377.
[2] Gorup-Besanez, "Lehrbuch," 1878, S. 401.
[3] Hoppe-Seyler, "Physiol. Chem.," 1881, S. 605.
[4] For analyses of pericardial fluid from the horse, and also for the analysis of various dropsical fluids which tend to accumulate in the serous cavities of man, see Halliburton, "Chem. Physiol.," pp. 338 and 339, and *Brit. Med. Journ.*, London, 1890.

liquid of specific gravity about 1007–8, and of a faintly alkaline reaction. It contains only about 1 per cent. of solids, chiefly inorganic salts, of which the greater part is sodium chloride, the other salts being potassium chloride, phosphates of lime and magnesia, and traces of iron and sulphates.[1] There is, as a rule, not more than 1 part per 1000 of proteids. These consist almost entirely of proteoses; chiefly in the form of protoalbumose, which is precipitable by saturation with sodium chloride or magnesium sulphate. There is also a very small amount of serum globulin, but no serum albumin or fibrinogen, nor is there any nucleo-proteid or fibrin ferment. Rarely peptone occurs.

In addition to proteids and traces of nitrogenous extractives, there is present in cerebro-spinal fluid a non-nitrogenous substance peculiar to it, which has the property of reducing copper salts when heated with them in an alkaline solution. This was thought by Claude Bernard to be sugar. The substance, however, is not sugar, being non-fermentable, non-rotatory, and incapable of combining with phenylhydrazin to form a crystalline compound. According to Halliburton, it is pyrocatechin, and has the formula $C_6H_4(OH)_2$, being probably one of the decomposition products of proteids; it occurs in traces in the urine. In tapped cases of hydrocephalus and meningocele the amount of this substance increases after the first tapping.[2]

The presence of proteoses, and occasionally of peptones, in the cerebro-spinal fluid, although these substances do not occur in blood or lymph, is of interest in connection with the theory of Gaskell, which supposes the central nervous system of vertebrates to have become developed in connection with a dorsal alimentary canal, such as is found in arthropods.[3] No digestive ferment (pepsin, trypsin) has, however, been detected in cerebro-spinal fluid.

Synovia differs from lymph in containing a larger amount of solids and also a mucin-like substance. Mucin, according to Landwehr,[4] yields a reducing sugar on boiling with mineral acids, but, according to Hammarsten,[5] this mucin-like substance of synovia does not yield such reducing sugar, and is of the nature of nucleo-albumin (containing 5 per cent. of phosphorus).[6] But the mucin-like material obtained by Salkowski[7] from synovia neither yielded phosphorus nor did it give any reducing sugar.

Salkowski gives the following as the composition of the synovia analysed by him:—

In 100 grms.—	Water	93·084	
,,	Mucin-like substance . .	0·375	5·199
,,	Other proteids . . .	4·824	
,,	Fat	·282	
,,	Lecithin	0·017	
,,	Cholesterin . . .	0·569[8]	
,,	Inorganic salts . . .	0·849 (Nacl 0·772)	

[1] Yvon, quoted by Halliburton.

[2] For further details consult Halliburton, "Chem. Physiol.," p. 355; also "Report of Spina Bifida Committee," *Trans. Clin. Soc. London*, vol. xviii.; and *Journ. Physiol.*, Cambridge and London, vol. x. p. 232, where the previous literature will be found.

[3] Address to the Section of Physiology, *Rep. Brit. Ass. Adv. Sc.*, London, 1896.

[4] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxxix. S. 193.

[5] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xii. S. 484.

[6] For analysis of synovia by different observers, see Halliburton, "Chem. Physiol.," p. 351.

[7] *Virchow's Archiv*, Bd. cxxxi. S. 304.

[8] This is unquestionably abnormally high. The fluid was from a case of chronic coxitis.

HÆMOGLOBIN: ITS COMPOUNDS AND THE PRINCIPAL PRODUCTS OF ITS DECOMPOSITION.

By Arthur Gamgee.

Contents.—Distribution in the Animal Kingdom, p. 186—Relations to other Constituents of Red Corpuscles, p. 188—Arterin? and Phlebin? p. 190—Oxyhæmoglobin, p. 193—Methods of obtaining, p. 194—Composition of, p. 197—Crystalline form, p. 203—Action of Reagents on, p. 207—Spectrum, p. 208—Spectrophotometry, p. 213—Photographic spectrum, p. 225—Hæmoglobin, p. 229—Preparation of, p. 232—Colour and Spectrum, p. 233—Compounds with Gases, p. 237—Derivatives and Products of Decomposition, p. 243.

By the term hæmoglobin[1] is designated the highly complex, iron-containing, crystalline colouring matter, which forms the most important constituent of the coloured corpuscles of the blood,[2] and in virtue of which they perform their function as the oxygen-carriers of the organism. This body possesses the remarkable property of linking to itself a molecule of oxygen, so as to form an easily dissociated compound, which is termed oxyhæmoglobin, to distinguish it specifically from the colouring matter which has parted with its dissociable oxygen; for the latter some retain the name *hæmoglobin*, though it is commonly, and by English writers usually, distinguished by the term *reduced hæmoglobin*.

Both oxyhæmoglobin and reduced hæmoglobin invariably (Hüfner) exist side by side in varying proportions in the living blood; the former being most abundant in arterial, the latter in venous, blood. In the present chapter the term hæmoglobin will be generally employed when speaking of the blood-colouring matter, without specific reference to its relation to oxygen; the term *reduced hæmoglobin* being invariably employed when reference is made to the colouring matter, deprived of its dissociable, or, as we may term it, in consideration of the part which it plays in the organism, its *respiratory* oxygen.

Should we speak of "hæmoglobin" or "the hæmoglobins," of "oxyhæmoglobin" or "the oxyhæmoglobins?"—In a subsequent section, it will be shown that the blood-colouring matter is by no means absolutely identical in all animals, but that it exhibits considerable variations in certain physical characters, and in chemical

[1] Hoppe-Seyler, to whom we owe a great part of our knowledge of the blood-colouring matter, first suggested this term. "Um Verwechselungen zu vermeiden nenne ich das Blutfarbstoff 'Hæmoglobulin oder Hæmoglobin,'" *Virchow's Archiv*, 1864, Bd. xxix. S. 223.

[2] Hæmoglobin constitutes about 40·4 per cent. of the weight of the moist corpuscles, and about 95·5 per cent. of all the organic substances contained in them.

composition, according to the species of animal from which it has been derived. Based upon these facts, or perhaps in order to emphasise them, it is now customary with German writers, following the example of Hoppe-Seyler, to speak, not of "oxyhæmoglobin," but of "the oxyhæmoglobins," of "the hæmoglobins" and not of "hæmoglobin." This appears to me to be an unnecessary and misleading attempt to attain accuracy in scientific terminology, at the expense of true and philosophical conceptions. As will be shown in the sequel, the proportion in which iron, the characteristic element in the blood-colouring matter, occurs, is absolutely the same in many animals, the weight of the molecule being probably identical in these cases. There is further abundant evidence in favour of the view that the typical nucleus, upon which the optical and physiological properties of hæmoglobin depend, is absolutely identical in all animals. The grounds for this assertion will be given in the sequel, when it will be shown that the opinion advanced of recent years, as to the existence of several hæmoglobins, not only varying in composition, but possessed of different powers of combining with oxygen, rests upon undoubted fallacies.

Distribution of Hæmoglobin throughout the Animal Kingdom.

After the discovery by Hoppe-Seyler [1] of the characteristic spectrum of hæmoglobin had enabled him definitely to prove that this substance is the true blood-colouring matter, Kühne [2] showed that the same body is the cause of the red colour of the voluntary muscles of vertebrates. Hunefeld [3] and Rollett [4] had shown that the blood of the earth-worm and of *Chironmous* yielded crystals identical with the blood crystals obtained from other animals; and Ray Lankester [5] and Nawrocki [6] simultaneously established the fact that these crystals consisted of hæmoglobin, by examining their spectroscopic characters.

In a series of researches, which extended from 1867 to 1872, Lankester investigated the distribution of hæmoglobin throughout the animal kingdom, and comparatively few facts have since been added to those which he published in 1872.[7]

The following are among the principal facts hitherto ascertained in relation to the distribution of hæmoglobin.[8]

Hæmoglobin occurs:—

1. In special corpuscles—

(*a*) In the blood of all vertebrates, excepting *Leptocephalus* and *Amphioxus*.

[1] Felix Hoppe in Tübingen, "Ueber das Verhalten des Blutfarbstoffes im Spectrum des Sonnenlichtes," *Virchow's Archiv*, 1862, Bd. xxiii. S. 446-449; "Ueber die chemischen u. optischen Eigenschaften des Blutfarbstoffs," *Virchow's Archiv*, 1864, Bd. xxix. S. 233-245.

[2] "Ueber den Farbstoff der Muskeln," *Virchow's Archiv*, 1865, Bd. xxxiii. S. 79; Kühne, "Lehrbuch d. phys. Chemie," 1868, S. 288.

[3] "Das Blut der Regenwürmer," *Journ. f. prakt. Chem.*, Leipzig, 1839, Bd. xvi. S. 152.

[4] "Zür Kenntniss der Verbreitung des Hæmatins," *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1861, Bd. xliv. S. 615-630.

[5] "Observations with the Spectroscope," *Journ. Anat. and Physiol.*, London, 1867, S. 114.

[6] "Optische Eigenschaften des Blutfarbstoffs," *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 196.

[7] "A Contribution to the Knowledge of Hæmoglobin," *Proc. Roy. Soc. London*, 1872, vol. xxi. pp. 70–81.

[8] The student is advised to read the interesting chapter, entitled "The Blood of Invertebrate Animals," in Halliburton's Text-Book, see pp. 316–330.

(In *Amphioxus* Lankester failed to obtain spectroscopic evidence of the presence of hæmoglobin, though Wilhelm Müller of Jena had described the corpuscles of this vertebrate as of a pale red colour.):

(*b*) In the perivisceral fluid of some species of the vermian genera, *Glycera*, *Capitilla*, and *Phoronis*:

(*c*) In the lamellibranchiate molluscs *Solen* and *Arca*.

2. Diffused in a vascular or ambient liquid—

(*a*) In the peculiar vascular system of the chætopodous annelids, very generally, but with apparently arbitrary exceptions:

(*b*) In the vascular system (which represents a reduced perivisceral cavity) of certain leeches (*Nephelis*, *Hirudo*), but not of all:

(*c*) In the vascular system of certain turbellarians, as in *Polia sanguirubra*:

(*d*) In a special vascular system (distinct from the general blood system) of a marine parasitic crustacean (undescribed), observed by Professor Edouard van Beneden:

(*e*) In the general blood system of the larva of the dipterous insect *Chironomus*; and in *Musca domestica*:[1]

(*f*) In the general blood system of the pulmonate mollusc *Planorbis*. Mr. H. C. Sorby expressed the opinion that probably the colouring matter found in the blood of *Planorbis* is not identical with hæmoglobin. I have shown, however, that the position of the absorption-bands of the colouring matter of the blood of *Planorbis* coincides exactly with that of the hæmoglobin bands:[2]

(*g*) In the general blood system of the crustaceans *Daphnia* and *Cheirocephalus* (Lankester); also in *Apus* and *Cypris*.[3]

3. Diffused in the substance of muscular tissue—

(*a*) In the voluntary muscles generally of Mammalia, and probably of birds, and in some muscles of reptiles:

(*b*) In the muscles of the dorsal fin of the fish *Hippocampus*, being generally absent from the voluntary muscular tissue of fish:

(*c*) In the muscular tissue of the heart of Vertebrata generally:

(*d*) In the unstriped muscular tissue of the rectum of man, being absent from the unstriped muscular tissue of the alimentary canal generally:

(*e*) In the muscles of the pharynx and odontophore of the gastropodous molluscs (observed in *Lymnæus*, *Paludina*, *Littorina*, *Patella*, *Chiton*, *Aplysia*), and of the pharyngeal gizzard of *Aplysia*, being entirely absent from the rest of the muscular and other tissues and the blood of these molluscs:

(*f*) In the muscular tissue of the pharyngeal tube of *Aphrodite aculeata* (Lankester), being absent from the rest of the muscular tissue, and from the blood in this animal, and absent from the muscular tissue generally in all other annelids, as far as yet examined.

4. Diffused in the substance of nervous tissue—

(*a*) In the chain of nerve ganglia of *Aphrodite aculeata* (Lankester). In this annelid the chain of nerve ganglia possesses a bright crimson colour. The colour is most intense in the supra-œsophageal ganglion, which has as intense a colour as a drop of fresh human blood. The colour impregnates the nerve itself, and is not contained in a liquid bathing the tissue:

(*b*) An exactly similar observation has been made by Hubrecht, who found hæmoglobin in the red-coloured cerebral ganglia of certain Nemertine worms, which possess no coloured blood corpuscles.[4]

[1] MacMunn, "Animal Chromatology," *Proc. Birmingham Phil. Soc.*, vol. iii. p. 130 (quoted at second-hand).

[2] Gamgee, "A Text-Book of the Physiological Chemistry of the Animal Body," vol. i. p. 131.

[3] Regnard et Blanchard, "Note sur la présence de l'hémoglobine dans le sang des crustacés branchiopodes," *Compt. rend. Soc. de biol.*, Paris, 1883, pp. 197–200.

[4] A. A. W. Hubrecht, "Untersuch. ueber Nemertinen aus dem Golf von Neapel," *Niederland. Arch. f. Zoologie*, 1876, Heft 3, Abstract in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. vi. S. 92.

The Probable Relations of the Blood-Colouring Matter to the other Constituents of the Coloured Corpuscles.

Without encroaching upon the domain of histology, reference must be made to the two principal views which have been advanced in reference to the structure of the coloured corpuscles.

According to the first,[1] which dates from the time of Bidloo,[2] Wells,[3] and Hewson,[4] and which was strongly advocated by Schwann, the coloured corpuscles of the blood are vesicular bodies, possessing an external envelope enclosing fluid contents.

This view has been revived and strongly insisted upon by Schäfer,[5] who briefly describes the structure of the red corpuscle in the following sentence:—"Each red corpuscle is formed of two parts, a coloured and a colourless, the former being a solution of *hæmoglobin*; the latter, the so-called stroma, which is in by far the smaller quantity, being composed of various substances, chief among these being lecithin and cholesterin, together with a small amount of cell globulin."[6]

According to the second view, which, in its present form, we owe to Rollett[7] and Brücke,[8] and which for many years found general favour, the coloured blood corpuscle is not considered as vesicular, but as a viscous solid mass composed of a colourless, highly elastic framework, *the stroma* (Rollett) denser at the periphery than at the centre, in the interstices or trabeculæ of which hæmoglobin and the other constituents of the corpuscles are contained.

Without attempting to decide which of these views, if either, is the correct one, it is expedient to consider some questions bearing upon them, and towards the solution of which we possess important facts.

Making for the moment the assumption which, as will be shown in the sequel, is denied by Hoppe-Seyler, that oxyhæmoglobin exists as such in the coloured blood corpuscles, the question arises, in what physical state does it occur? Is it simply dissolved in the liquid contents of the corpuscles, or is it dissolved in virtue of its being in combination with other constituents? Is it in a solid condition? and if so, is there any evidence as to whether its structure is crystalline or amorphous?

That the colour of the blood does not depend upon a simple *aqueous* solution of hæmoglobin, is evident when we consider that the blood corpuscles are among the soft parts of the body which contain the least water;[9] and that not only is the water which the coloured corpuscles contain altogether insufficient to hold the hæmoglobin in solution, but in some animals, the hæmoglobin of which is more sparingly soluble than

[1] A reference to and discussion of the earlier literature relating to this view will be found in Gamgee's "Physiological Chemistry," vol. i. p. 72.

[2] "Anatomia humani corporis, 1685," quoted by Milne-Edwards, "Leçons, etc.," tome i. p. 66.

[3] "On the Colour of the Blood," *Phil. Trans.*, London, 1797, p. 429.

[4] Hewson's Works, *Syd. Soc.*

[5] "Quain's Anatomy," 1891, vol. i. pt. 2, p. 210.

[6] Halliburton and Friend; since shown to be a nucleo-proteid.—Editor.

[7] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlvi. Abth. 2, S. 73.

[8] Brücke applied the term *Oekoid* to the stroma, *ibid.*, Wien, 1867, Bd. lvi. Abth. 2, S. 79.

[9] According to Bunge, "Zur quantitativen Analyse des Blutes," *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 191, the blood corpuscles contain 36·7 parts of solids, and 63·3 parts of water; muscular tissue contains about 25 per cent. of solids, and 75 per cent. of water; nerves contain about 22 per cent. of solids, and 78 per cent. of water.

is generally the case, the whole of the water contained in the blood would not suffice to dissolve the hæmoglobin stored up in the coloured corpuscles.

That the hæmoglobin is not contained in the blood corpuscles in the form of infinitely minute crystals, is proved by examining the corpuscles between crossed Nicols, when they are found not to be doubly refracting; whilst crystals of hæmoglobin, even when reduced to a state of most minute subdivision, are so.[1]

Furthermore, no crystalline or granular structure can be discovered when the coloured corpuscles are examined with the highest powers of the microscope.

The assumption was made by Preyer,[2] that hæmoglobin exists in the corpuscles in combination with potassium, alkaline solutions possessing the property of dissolving much larger quantities of hæmoglobin than pure water, and potassium being the most abundant of the mineral constituents of the coloured corpuscles of man, *though by no means of all animals.*[3] But, as a matter of fact, the coloured blood corpuscles do not behave as if they contained free hæmoglobin in a solid condition, or in solution, or a solution of an alkaline compound of hæmoglobin. Only one proof of this statement need be given in this place, others being adduced when discussing the remarkable, and, as it appears to me, untenable proposition of Hoppe-Seyler, that the blood-colouring matter, as it exists in the living corpuscles, differs remarkably in properties from hæmoglobin, so that it should be distinguished by a separate name or names. The one proof to which reference is made is furnished by the fact that the colouring matter of the red corpuscles is not extracted from them by the plasma, *or serum, or by fairly concentrated solution of neutral salts*, as would be the case if they contained free hæmoglobin, or an alkaline compound of that substance.[4] To explain the fact that hæmoglobin is retained by the corpuscles, Hoppe-Seyler advanced the plausible hypothesis, that it exists in them in combination with some constituent of the stroma, and he expressed the opinion that this constituent is lecithin. There are absolutely no grounds for the latter assumption; and it has indeed yet to be proved that the phosphorus-containing principle of the stroma of the coloured corpuscles is lecithin and not protagon, as had been very positively asserted by Hermann.[5]

Without attempting to speculate beyond the facts which we possess, it may, however, be assumed that hæmoglobin exists in the blood corpuscles in the form of a compound with a yet unknown constituent of the corpuscle. This compound, the existence of which we are forced to assume, is characterised by remarkable instability, for it is decomposed, setting free the hæmoglobin, which then passes into solution—(1) when the blood plasma or serum, in which the corpuscles are suspended, is diluted; (2) when certain substances act upon the corpuscles (ether, chloroform, salts of the bile acids, certain products of putrefaction); (3) by the action of heat; by alternate freezing and thawing; by induction shocks, etc.

[1] W. Preyer, "Die Blutkrystalle," Jena, 1871, S. 28.

[2] *Ibid.*, S. 30–33.

[3] G. Bunge, "Zur quantitativen Analyse des Blutes," *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 191.

[4] This is no real proof that hæmoglobin is not in solution; it is merely a statement of the fact that it is indiffusible through the unaltered envelope of the corpuscle. It is, moreover, capable of proof that the contents of the red corpuscles are completely fluid during life. Cf. p. 193, lines 9 to 12.—EDITOR.

[5] *Arch. f. Anat. u. Physiol.*, Leipzig, 1866, S. 33.

Hypothesis of Hoppe-Seyler, that the coloured substance of the corpuscles possesses properties which differ from those of hæmoglobin—Arterin (?), and Phlebin (?).—It has been shown that we are forced to assume the existence in the coloured corpuscles of a very unstable compound of hæmoglobin. Hoppe-Seyler, as far back as 1877,[1] expressed the opinion that whilst the compound or compounds of hæmoglobin existing in the blood corpuscles absorb the rays of the spectrum precisely as solutions of hæmoglobin, in other respects very remarkable differences can be detected, certain of these differences being, in his opinion, of great physiological importance.

Subsequently,[2] Hoppe-Seyler, returning to this subject, endeavoured to prove by a variety of arguments that such are the differences between the properties of the colouring matter as it exists in the coloured corpuscles and pure hæmoglobin, that we cannot logically assert that they are identical. He examined in detail the differences in behaviour which had been observed by himself and by others, between the blood-colouring matter as it exists in the corpuscles and solutions of pure oxy- or reduced hæmoglobin. He referred to the undoubted fact that the colouring matter, as it exists in the coloured corpuscles, is not dissolved out by serum, liquor sanguinis, or saline solutions, of a certain strength. It does not, he alleged, crystallise, nor readily yield its dissociable oxygen when heated *in vacuo*; it readily decomposes peroxide of hydrogen (H_2O_2), setting free ordinary inactive oxygen, and is not oxidised during the process; a solution of potassium ferricyanide does not for a long time attack the blood corpuscles, or convert their colouring matter into methæmoglobin.

On the other hand, a solution of oxyhæmoglobin (or, as Hoppe-Seyler preferred to express it, of *the oxyhæmoglobins*, so as to recall the fact of the minor differences presented by the hæmoglobin of different species of animals) is soluble in serum or in blood plasma, or in solutions of the neutral salts; it crystallises with greater or less facility, according to the animal whence the blood is obtained. When thoroughly pure, it has scarcely any power of decomposing H_2O_2, but under the influence of this body it is readily oxidised.

Solutions of crystallised oxyhæmoglobin, Hoppe-Seyler maintained, give up their dissociable oxygen with difficulty and incompletely, when heated *in vacuo*. When blood is saturated with CO, this gas can subsequently be entirely removed, by passing a stream of hydrogen gas through it for some hours, or by long-continued boiling *in vacuo*. On the other hand, when a solution of oxyhæmoglobin is saturated with CO, and the solution is heated *in vacuo*, the poisonous gas is, Hoppe-Seyler stated, given off with great difficulty and incompletely.

Lastly, highly dilute solutions of potassium ferricyanide readily convert the oxyhæmoglobins into methæmoglobin.

The evidence by which Hoppe-Seyler endeavoured to prove that the properties of the blood-colouring matter, as it exists in the corpuscles, differ so greatly from those of hæmoglobin, that we cannot with truth say that this body exists in them, is, on every single point, of so unsatisfactory a character as not to stand a moment's investigation, and would lead us to reject his hypothesis, even if we had not been placed in possession of some remarkable facts bearing on this subject, which have been ascertained by the method of spectrophotometry. The non-crystallisation of the colouring matter as it exists in the coloured corpuscles might, were it really true, well be explained by the fact that hæmoglobin does not exist in a free state, but is combined

[1] "Physiologische Chemie," Berlin, 1877, Th. 1, S. 381.

[2] "Beiträge zur Kenntniss des Blutfarbstoffes," *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 477.

with another constituent of the corpuscle; but the statement itself, as made by Hoppe-Seyler, is incorrect. Although the fact has been denied by some writers, there can be no question whatever, on the evidence of so eminent an observer as Kühne,[1] as well as of Funke,[2] Brisegger and Bruch,[3] Böttcher, Kölliker, L. Beale,[4] Owsjannikow,[5] Richardson,[6] and Klebs, that what Preyer terms "intraglobular crystallisation" can and does occur, *i.e.* a single crystal forms in the interior of a coloured blood corpuscle. The process is most easily followed in the blood corpuscles of certain fishes,[7] though it has also been observed in those of the dog (Kühne) and of the rat.[8] The most remarkable fact with regard to intraglobular crystallisation is, that when water is added to a preparation exhibiting it, the crystal at once disappears, and the corpuscles resume their original appearance.[7]

Again, at first sight, the difference in behaviour of the blood corpuscles and of hæmoglobin towards peroxide of hydrogen appears thoroughly in favour of Hoppe-Seyler's hypothesis. It was, however, shown by Bergengruen, who first discovered the facts in reference to H_2O_2, that the decomposing action exerted by the blood corpuscles on H_2O_2 depends upon their stroma. Solutions of perfectly pure crystals of oxyhæmoglobin have no action whatever on peroxide of hydrogen, whilst the stroma of the coloured blood corpuscles exerts an intense action.[8] All forms of protoplasm (splenic cells, colourless corpuscles, yeast cells), decompose H_2O_2, though the stroma of the coloured corpuscles acts most powerfully. The fact of the decomposing action being exerted by the stroma, and the stroma only, explains why the blood corpuscles are not oxidised whilst oxyhæmoglobin is so, the colouring matter in the corpuscles not coming in contact with the undecomposed H_2O_2.

The greater readiness, as compared with pure hæmoglobin, with which, according to Hoppe-Seyler, the blood corpuscles give up either the oxygen or the carbonic oxide which may be combined with their colouring matter (if the facts were true, which we are not prepared to admit), would be much more probably due to a katalytic action, exerted by some other constituent of the corpuscle, than to any radical difference between the colouring matter of the corpuscles and hæmoglobin.

The one point of difference between the colouring matter of the corpuscles and oxyhæmoglobin, which at first sight appears most difficult to explain, is the action of solution of potassium ferricyanide. As von Mering[9] showed, if fresh defibrinated blood be mixed with solutions containing $2\frac{1}{2}$, 5, and 10 per cent. of the ferricyanide, the mixture assumes a scarlet colour, and even after twenty-four hours contains the blood-colouring matter unaltered. On adding, however, the same solution of the ferricyanide, in the same proportions, to solutions of pure oxyhæmoglobin, they assume *almost instantaneously* the colour, and exhibit the spectrum of methæmoglobin.

[1] W. Kühne, *Virchow's Archiv*, Bd. xxxiv. S. 423.

[2] "Ueber Blutkrystallisation," *Ztschr. f. rat. Med.*, 1852, N.F., Bd. i. S. 288–292.

[3] "Blutkrystalle, und organische Krystalle ueberhaupt" (15th Sept. and 15th Oct. 1852), *Verhandl. d. naturf. Gesellsch. in Basel*, 1854–1857, Bd. i. S. 173–185.

[4] "Observations upon the Nature of the Red Blood Corpuscles." *Quart. Journ. Micr. Sc.*, London, 1864, pp. 32–43.

[5] "Zur Histologie der Blutkörperchem," *Bull. Acad. d. sc. de St. Pétersbourg*, vol. viii. pp. 561–572 (describes intraglobular crystallisation in *Osmerus eperlanus*).

[6] "Structure of the Red Blood Corpuscles," Philadelphia, 1870 (describes intraglobular crystallisation in *Menobranchus*).

[7] Kühne, see W. Preyer, "Die Blutkrystalle," Jena, 1871, S. 2 and 3. See also Funke's "Atlas of Physiological Chemistry" (London, printed for the Cavendish Society, 1853), Plate x. fig. 5, and the description at p. 17 of the "Description of the Plates."

[8] Paul Bergengruen, "Ueber die Wechselwirkung zwischen Wasserstoffsuperoxyd und verschiedenen Protoplasmaformen," Inaug. Diss., Dorpat, 1888.

[9] "Ueber die Wirkung des ferricyan. Kalium auf Blut," *Ztschr. f. physiol. Chem.*, Strassburg, 1883, Bd. viii. S. 186, 189.

The extraordinary difference in the result predisposes one at first to conclude that it must be due to a radical difference betwen the colouring matter of the corpuscles and oxyhæmoglobin, such as Hoppe-Seyler believed to exist. If, however, instead of the solutions mentioned above, solutions ten times more dilute (containing 0·25, 0·5 and 1 per cent.) be mixed with defibrinated blood in the same proportions as before, the mixture assumes instantly the colour, and exhibits the spectrum of methæmoglobin. In this case the dilute solution extracts, in the first instance, the blood-colouring matter from the corpuscle, and then the ferricyanide acts upon the solution, exactly as it does when brought in contact with a solution of crystals of oxyhæmoglobin. The fact that the strong solution of potassium ferricyanide does not act upon the colouring matter of the blood corpuscles, is due to its incapacity to reach, in the first instance, the oxyhæmoglobin of the corpuscles. In this case also, it appears that the difference (supposed) between the colouring matter, as it exists in the intact blood corpuscles and solutions of hæmoglobin, is only an apparent one.

Though closely connected with the subject which has been discussed in this section, the views of Bohr[1] (who believes that he has succeeded in establishing, in addition to the already known oxyhæmoglobin, the existence of at least three additional compounds of oxygen with hæmoglobin, all possessing the spectrum of oxyhæmoglobin, but differing in elementary composition and in their capacity to combine with oxygen), will be referred to under the heading of "Oxyhæmoglobin." There can be no question, however, that these views have been completely disproved by Hüfner,[2] the supposed individual oxyhæmoglobins of Bohr being mechanical mixtures of pure oxyhæmoglobin with products of its decomposition,—the necessary results of the methods of preparation followed by the Scandinavian observer.

We have shown that even admitting, for the sake of argument, the correctness of all Hoppe-Seyler's statements, these when carefully analysed afford no evidence whatever in support of his bold hypothesis. Whilst such is the case, the splendid investigations of Hüfner[2] have conclusively proved that, in respect of its power of combining with oxygen, the blood-colouring matter, as it exists in the coloured blood corpuscles, behaves precisely as a solution of pure hæmoglobin of the same concentration. Further, by the method of spectrophotometry, Hüfner has shown, as could be done by no other method, that the colouring matter of the blood *is one*—hæmoglobin—and that in every specimen of living blood, this colouring matter exists, partly as oxyhæmoglobin and partly as reduced hæmoglobin.

The discussion which has preceded will have prepared the reader for the conclusion, which appears to be the only one which can legitimately be based upon the facts in our possession—to wit, that whilst oxyhæmoglobin and reduced hæmoglobin exist in the coloured blood corpuscles in the form of loose or unstable combinations with some other constituent of the corpuscle, evidence is altogether wanting in support of Hoppe-Seyler's contention that the blood-colouring matter, as it exists in the corpuscles, possesses properties so different from those of oxyhæmoglobin and of reduced hæmoglobin, as to warrant its being looked upon as a distinct substance, to be distinguished by a different name. Hoppe-Seyler suggested,[3] indeed, that the colouring matter of arterial blood should be called *Arterin*, to distinguish it from oxyhæmoglobin, whilst that contained in venous blood should be named *Phlebin*, to

[1] "Ueber die Verbindungen des Hämoglobins mit Sauerstoff," "Ueber die specifische Sauerstoffmenge des Blutes und die Bedeutung derselben für den respiratorischen Stoffwechsel," *Centralbl. f. Physiol.*, Leipzig u. Wien, 1890, Bd. iv. S. 242, 254.

[2] In this place it is only necessary to refer to one of Hüfner's papers. See G. Hüfner, "Neue Versuche zur Bestimmung der Sauerstoffcapacität des Blutfarbstoffs," *Arch. f. Physiol.*, Leipzig, 1894, S. 130, 176. Refer particularly to pp. 130, 134, 175, 176.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 495.

distinguish it from reduced hæmoglobin! The only distinction which Hoppe-Seyler found to exist between "arterin" and "phlebin" consisted in the alleged greater ease with which the hypothetical constituent of arterial blood yielded its dissociable oxygen, when boiled *in vacuo*, as compared with the hypothetical constituent of venous blood. To establish this alleged difference between arterial and venous blood would require a body of experimental facts, such as does not exist. Even were the difference shown to be a real one, it would in no way support the hypothesis of a radical difference between the colouring matter of arterial and venous blood. But the investigations of Hüfner, which have proved with mathematical accuracy that the colouring matter of the blood behaves, both in so far as its optical characters and its relations to oxygen are concerned, precisely as a solution of hæmoglobin, and is *the only coloured constituent of the corpuscles*, complete the demonstration of the erroneous nature of the hypothesis advanced by Hoppe-Seyler on this subject.

OXYHÆMOGLOBIN.

Methods of Preparation.

Introductory remarks.—It has already been stated that the blood-colouring matter of different species of animals is not, in all particulars, absolutely identical. Although behaving in the same manner in reference to the gases with which it can combine to form more or less easily dissociated compounds, and whilst possessing identical powers of absorbing the rays of the spectrum, the hæmoglobin of different animals exhibits differences (1) in crystalline form, (2) in solubility, (3) in the quantity of water of crystallisation, (4) in percentage composition. These differences will be carefully examined in the sequel, but attention is drawn to them in this place, in relation to another point of difference, namely, the variation in the facility of separating hæmoglobin in a crystalline form. From the blood of certain animals, crystals of hæmoglobin can most readily be prepared, whilst in other cases the task is one of very considerable difficulty. Among the conditions which influence the result, the degree of solubility of the blood-colouring matter is the chief. Thus the blood of the rat, the guinea-pig, and the squirrel, which contains the least soluble hæmoglobin, yields crystals with great facility; whilst the blood of man, and that of the domestic herbivorous animals, which possess hæmoglobin of remarkable solubility, yields crystals with extraordinary difficulty. It is impossible to state with accuracy the relative facility of crystallisation of the hæmoglobin of different animals, but the following statements are probably correct. The blood of the rat, the guinea-pig, and the squirrel crystallises most readily; next comes the blood of the cat, the dog, and the horse; the blood of man and the pig follow, whilst that of the rabbit, the sheep, the ox, and the frog crystallise with the greatest difficulty.

The principle upon which the majority of the methods for the separation of hæmoglobin in a crystalline form are based is the following:—To effect the solution of the hæmoglobin of the coloured corpuscles in the serum, or in water, added to the previously separated corpuscles; and thereafter, by the addition of alcohol, or of ether, or by the agency of cold, or of both cold and alcohol or ether conjointly, sometimes aided by the process of evaporation, to cause the hæmoglobin,

which is sparingly soluble in dilute alcohol, especially at low temperatures, to crystallise. In the case of animals, the hæmoglobin of whose blood is very sparingly soluble, the addition of alcohol or ether is often dispensed with. We shall, in the first place, describe those methods which readily furnish hæmoglobin crystals for the purposes of microscopical research, and then the methods which are employed for the preparation and purification of large quantities of hæmoglobin.

Methods employed in preparing small quantities of hæmoglobin for microscopic examination.—1. *Funke's method.*[1]—From the blood of those animals whose blood crystallises readily, but especially in the case of the rat, oxyhæmoglobin can be obtained for microscopic examination in three or four minutes, by receiving a drop of blood on a glass slide, adding a drop of distilled water, mixing the two liquids by means of a needle, and spreading the mixture over the central part of the slide. When the edges of the liquid commence to dry, cover with a microscopic covering glass. Crystals of hæmoglobin form at once.

2. *Rollett's method.*[2]—A platinum capsule is placed in a freezing mixture, and freshly defibrinated blood is poured into it, so as to convert it into a lump of red ice. After being in the freezing mixture for half an hour the blood is allowed to thaw gradually, and the contents of the capsule are poured into a glass vessel of such dimensions that the bottom is covered by the lake-coloured blood to a depth of 15 mm.; the glass vessel is then set aside in a cool place. In a short time, the blood of rats, of guinea-pigs, and of squirrels, treated by this method, furnishes well-formed crystals.

3. *Gscheidlen's method.*[3]—Defibrinated blood, which has been exposed to the air for a period of twenty-four hours, is sealed in narrow glass tubes (vaccine tubes answer well), and these tubes are then placed in the incubator and kept a temperature of about 37° C. for some days. On opening the tubes and emptying their contents into a watch glass or on a glass slide, and allowing some time for evaporation to take place, crystals of extraordinary size are obtained.

4. *Max Schultze's method.*[4]—Defibrinated blood is heated (on a warm stage, in the case of a microscopic preparation) to a temperature of 60° F., when the corpuscles dissolve and the blood becomes lake-coloured; it is then allowed slowly to cool and to evaporate. This method may be employed with large quantities of blood, and Preyer[5] found that by no other method did he obtain as fine and as large crystals from horse's blood.

In addition to the four methods which have been above described as most conveniently yielding crystals of oxyhæmoglobin, when these are desired on a small scale, there are many others which have been employed, and which occasionally give good results.

Thus Rollett[6] found that when induction shocks were passed through blood, it became lake-coloured and yielded crystals of hæmoglobin, and

[1] *Ztschr. f. rat. Med.*, 1851, S. 185.

[2] "Versuche und Beobachtungen am Blute," *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1863, Bd. xlvi. S. 77.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvi. S. 421.

[4] "Ein heitzbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes," *Arch. f. mikr. Anat.*, Bonn, 1865, Bd. i. S. 31.

[5] "Die Blutkrystalle," S. 23.

[6] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1852, Bd. xlvi. S. 75.

A. Schmidt[1] made the same observation in reference to the blood near the positive pole, when this liquid is subjected to the action of a constant current.

Without undergoing any other treatment, the blood of asphyxiated animals often crystallises. Blood which has been deprived of its gases, by boiling *in vacuo*, often crystallises. Indeed, by this method, Preyer[2] succeeded in the very difficult task of crystallising sheep's blood.

Methods of preparing considerable quantities of oxyhæmoglobin.—Certain of the methods already recommended for the preparation of hæmoglobin, when small quantities only are needed for the purposes of microscopic investigation, might be employed in the preparation of larger quantities. Other processes, however, are to be preferred, and of these some which are specially to be recommended are given below. Of these processes, the first, or Hüfner's modification of it, should, by preference, be employed, especially if a preparation, as free as possible from products of decomposition, be desired.[3]

Hoppe-Seyler's method.[4]—Defibrinated blood is mixed with ten times its volume of a solution of sodium chloride[5] (made by diluting one volume of a saturated solution of NaCl with nine volumes of water), and the mixture is poured into shallow basins, which are set aside in a cool place, so as to allow the greater part of the blood corpuscles to settle.[6] The supernatant liquid is decanted, and the magma of corpuscles, mixed with a small quantity of water, is poured into a stoppered separating funnel. The contents of this funnel are treated with an equal volume of ether. After repeated, but not too violent, agitation, the deep red aqueous solution is separated from the supernatant ether, and filtered as quickly as possible. The clear red filtrate cooled to 0° C. is then mixed with one-fourth of its volume of absolute alcohol, likewise cooled to 0° C. The mixture is then maintained for a couple of days (and, if crystallisation has not occurred, even longer), at a temperature of $-5°$ C. to $-10°$ C. In a period varying between twenty-four and forty-eight hours crystallisation has usually occurred, and, unless the solution of hæmoglobin was too dilute, the whole of the liquid has set into a mass of crystals. The crystals are now collected on a filter (the process of filtration being carried on at as low a temperature as possible, in any case below 0° C.) and washed several times with a previously cooled mixture, composed of one volume of absolute alcohol and four volumes of distilled water. The filter with its contents is now placed between sheets of filtering paper, and as much as possible of the adhering mother-liquor is removed by gentle pressure. The oxyhæmoglobin thus

[1] "Zur Krystallisation des Blutes," *Virchow's Archiv*, 1864, Bd. xxix. S. 29.

[2] "Die Blutkrystalle," S. 19 and 20.

[3] The blood of the dog, and especially of the horse, are to be preferred for the preparation of large quantities of oxyhæmoglobin. As the success of the various operations depends upon their being conducted at a low temperature, the preparation of hæmoglobin for purposes of research should only be attempted in the depth of winter.

[4] "Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere," *Med. Chem. Untersuch.*, Berlin, 1866, S. 170, 180–185; "Handbuch d. physiologisch. chem. Analyse," Berlin, 1893, Aufl. 6, S. 274.

[5] In the preparation of hæmoglobin from the blood of birds, amphibia, and fish, sodium sulphate is to be employed in the place of sodium chloride. In the case of mammalian blood, it presents no advantages over sodium chloride.

[6] Instead of allowing the corpuscles to separate, as described, it is preferable to employ a centrifugal machine. The separation of the corpuscles from the mixture of serum and salt solution is not only very much more rapid, but also much more complete, and therefore the obtaining of pure oxyhæmoglobin is facilitated.

obtained may now be purified by being recrystallised. With this object the moist crystals are removed by means of a spatula from the filter, and placed in a flask or beaker, and about three times their volume of distilled water is added. The mixture is heated to 55° C., the solution filtered; the filtrate is cooled to 0° C., and to every four volumes one volume of absolute alcohol, cooled to 0° C., is added. The mixture is then cooled to −5° C. or −10° C.

When the oxyhæmoglobin separates again, this process of crystallisation may be repeated five or even six times, providing the temperature at which the various operations are conducted be a very low one. The recrystallised hæmoglobin obtained by these processes may be employed to make standard solutions of the body, or it may be dried. It is very questionable, however, whether the recrystallising of oxyhæmoglobin is advisable, for reasons to be stated below, it being probably better to purify the crystals by repeated washings with ice-cold water. Hoppe-Seyler states that oxyhæmoglobin can only be dried, without decomposition, *in vacuo*, at a temperature under 0° C. If dried at a higher temperature it assumes a dark colour, and ceases to be entirely soluble in distilled water.

Zinoffsky,[1] who worked with oxyhæmoglobin prepared from the blood of the horse, found that, when spread out in very thin layers, it could be dried *in vacuo* in eight hours, without undergoing decomposition, at a temperature of 10° C. to 20° C. He found that the oxyhæmoglobin thus prepared was entirely soluble in distilled water, and that the solution was not precipitated by lead acetate; proving that no methæmoglobin had been formed.

Hæmoglobin which has been dried *in vacuo*, over sulphuric acid or phosphoric anhydride, at a temperature of 0° C., may be heated to 110° C. or 115° C., without undergoing any decomposition.

Modifications of Hoppe-Seyler's method.—(*a*) Among numerous modifications may be mentioned one employed by Hüfner,[2] and which may with advantage be adopted in laboratories provided with centrifugal machines. The blood is not treated with salt solution, but the corpuscles are separated by the action of the centrifuge alone. Crystals thus obtained are treated with ice-cold water, separated by the centrifuge, and this process repeated several times. Finally, the crystals are dried on porous plates made of cellulose, or solutions are made of the yet moist crystals, and the percentage of hæmoglobin in them determined.

(*b*) The defibrinated blood of the dog is mixed with its own volume of distilled water, and the diluted fluid is treated with one-fourth its volume of alcohol. The mixture is kept for twenty-four hours, at a temperature which must be lower than 0° C. The crystals which separate are dissolved in about three times their bulk of distilled water, at a temperature of 30° C., and the solution being cooled to 0° C., a fourth of its volume of absolute alcohol at 0° C. is added. The fluid should be kept in a freezing mixture at a temperature of −10° C. to −20° C. for twenty-four hours. The whole fluid then becomes converted into a magma of crystals. The process of recrystallisation may be several times repeated.

[1] "Ueber die Grösse des Hämoglobin-moleculs," *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 15–34. See "Darstellung des Hämoglobins," S. 18–24.

[2] "Beitrag zur Lehre von Blutfarbstoffe," *Beitr. z. Physiol. C. Ludwig z. s. 70 Geburtst. etc.*, Leipzig, 1887, S. 74–81; and "Neue Versuche, u.s.w.," *Arch. f. Physiol.*, Leipzig, 1894, S. 134–136.

(*c*) Defibrinated blood is treated with about one-sixteenth its volume of ether (say 31 c.c. of ether to 500 c.c. blood), and the mixture shaken for some minutes. It is then set aside in a cool place. After a period, varying from twenty-four hours to three days, the liquid has been converted into a thick magma of crystals. These may be separated by placing in tubes and using the centrifugal apparatus. The cakes of crystals are treated with a mixture of one part of absolute alcohol and four parts of distilled water, and again centrifugalised. By repeating this process the crystals are ultimately obtained free from serum albumin. The crystals may be dissolved in water and recrystallised, as described in Hoppe-Seyler's method.

In addition to the methods described, many others have been suggested, and to these only a passing reference need be made.

Thus Kühne devised a method based upon the fact that the stroma of the coloured corpuscles is dissolved by the addition of a watery solution of crystallised bile (a mixture of sodium glycocholate and taurocholate).[1] Hüfner[2] and his pupil Otto employed a 1 per cent. alcoholic solution of chinoline, or a watery solution of the hydrochlorate of the same base, to prepare oxyhæmoglobin from pig's blood, though Otto afterwards found[2] that, by taking special precautions, Hoppe-Seyler's method is available, even in the case of pig's blood, and indeed preferable to all others.

Remarks on the purification of hæmoglobin.—It has, until lately, been assumed that in the preparation of pure oxyhæmoglobin the body should be recrystallised as frequently as possible, with the object of getting rid of all traces of adherent albuminous and saline impurities derived from the plasma or serum. Since spectrophotometry has supplied us with a method of determining, with an accuracy previously unattainable, the purity of a colouring matter, it has been found that although oxyhæmoglobin which has been recrystallised, when examined in the ordinary manner, exhibits a spectrum which appears identical with that of the colouring matter which has been only once crystallised, its spectrophotometric constants have changed; in other words, when oxyhæmoglobin is recrystallised it undergoes a change, possibly only affecting its physical, but more probably affecting its chemical constitution also. The knowledge of these facts has caused Hüfner in his recent researches to employ hæmoglobin which has not been recrystallised.

If precautions are taken in the first instance to separate (by the most perfect filtration, followed by prolonged centrifugalising) all formed elements and accidental solid impurities from the solution of blood corpuscles which is to be crystallised, and if the crystalline mass of oxyhæmoglobin obtained be repeatedly, say five or six times, treated with ice-cold water, the resulting solution being each time separated from the undissolved crystals by very rapid and very prolonged centrifugalising, the portion of the original crystals still left undissolved will be found, on chemical, microscopical, and spectrophotometric investigation, to furnish evidence of being a pure substance.

The new method is more easily and much more expeditiously carried out than the old.

Elementary composition of oxyhæmoglobin dried at 110°–115° C.—Before describing either the physical or chemical properties of the

[1] *Centralbl. f. d. med. Wissensch.*, Berlin, S. 833.

[2] The account of Hüfner's discovery of this method is contained in a paper by his pupil, F. Otto, "Ueber das Oxyhämoglobin des Schweines," *Ztschr. f. physiol. Chem.*, Strassburg, 1882–83, Bd. vii. S. 57.

blood-colouring matter, it is advisable to consider its elementary composition, and to ascertain how the results of chemical analysis bear on the question as to hæmoglobin being a definite chemical individual, its composition being invariable.

Hæmoglobin is a compound of carbon, hydrogen, nitrogen, sulphur, iron, and oxygen. The crystals of hæmoglobin contain water of crystallisation, which varies considerably in amount in the hæmoglobin of different animals. When ignited, pure hæmoglobin obtained from mammalian blood yields an ash composed entirely of ferric oxide; the hæmoglobin of birds and fishes, and probably of all animals with nucleated corpuscles, yields on ignition an ash which, in addition to Fe_2O_3, contains phosphoric anhydride (P_2O_5), derived in all probability from nuclein contained in the corpuscles.[1]

The earlier analyses of oxyhæmoglobin made by C. Schmidt[2] and by Hoppe-Seyler[3] exhibited results which appeared to indicate that crystallised oxyhæmoglobin is a body of constant composition. From the analyses of these two observers, and his own determinations of the iron and sulphur in crystallised oxyhæmoglobin, Preyer deduced the following as the mean percentage composition of oxyhæmoglobin:—

	In 100 Parts.
C	54·00
H	7·25
N	16·25
Fe	0·42
S	0·63
O	21·45
	100·00

On the assumption, which a large number of facts have since shown to be almost certainly correct, that the molecule of hæmoglobin contains one atom of iron, Preyer assigned to it the empirical formula $C_{600}H_{960}N_{154}FeS_3O_{170}$, the molecular weight being 13,332.

Analysis of Oxyhæmoglobin dried at a temperature above 100° *C.*[3] (Hoppe-Seyler).

Oxyhæmoglobin of	Water of Crystallisation in the Crystals which had been dried *in vacuo*.	C.	H.	N.	O.	S.	Fe.	P_2O_5.
Dog . . .	3-4 per cent.	53·85	7·32	16·17	21·84	0·39	0·43	...
Goose . . .	7 ,, ,,	54·26	7·10	16·21	20·69	0·54	0·43	0·77
Guinea-pig . .	6 ,, ,,	54·12	7·36	16·78	20·68	0·58	0·48	...
Squirrel . .	9 ,, ,,	54·09	7·39	16·09	21·44	0·40	0·59	...

[1] Y. Inoko, "Einige Bemerkungen ueber phosphorhaltige Blutfarbstoffe," *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xviii. S. 57.

[2] "Analyse der Blutkrystalle," in Böttcher's monograph, "Ueber Blutkrystalle," Dorpat, 1862.

[3] "Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere; Zusammensetzung der Farbstoffkrystalle des Meerschweinchen- und des Hunde-blutes," *Med. Chem. Untersuch.*, Berlin, 1868, S. 186 *et seq.*

The subsequent researches of Hoppe-Seyler soon demonstrated, however, that the blood crystals obtained from the blood of different animals did not possess an identical composition, though the differences brought out by Hoppe-Seyler's analyses were not very great. His results are shown in the table on p. 198.[1]

The very numerous analyses of oxyhæmoglobin of different animals, made in recent years by Kossel,[2] Otto,[3] Zinoffsky,[4] Hüfner,[5] Jaquet,[6] and others exhibit, however, such extraordinary discrepancies in the results of ultimate organic analysis as to preclude a precise answer being given to such simple questions as the following:—

Is hæmoglobin a body, having a constant composition in animals of the *same species*?

Does the hæmoglobin of *different animals* vary in chemical composition, and if so, within what limits?

Results of the more recent Analyses of Oxyhæmoglobin (1878–1890).

Oxyhæmoglobin of	C.	H.	N.	S.	Fe.	O.	P_2O_5.	
Dog . . .	53·85	7·32	16·17	0·39	0·43	21·84	...	Hoppe-Seyler.[7]
,, . . .	53·91	6·62	15·98	0·540	0·333	22·62	...	Jaquet.[8]
,, . . .	54·57	7·22	16·38	0·568	0·336	20·93	...	Jaquet.[9]
Horse . .	54·87	6·97	17·31	0·650	0·47	19·73	...	Kossel.[10]
,, . . .	54·76	7·03	17·28	0·67	0·45	19·81	...	Otto.[11]
,, . . .	54·40	7·07	17·40	0·66	0·45	19·74	...	Bücheler.[12]
,, . . .	51·15	6·76	17·94	0·39	0·335	23·43	...	Zinoffsky.[13]
Ox . . .	54·66	7·25	17·70	0·447	0·400	19·54	...	Hüfner.[14]
,, . . .	...	...	...	...	0·336	...	...	Hüfner.[15]
Pig . . .	54·17	7·38	16·23	0·660	0·430	21·360	...	Otto.[16]
,, . . .	54·71	7·38	17·43	0·479	0·399	19·602	...	Hufner.[17]
Hen. . .	52·47	7·19	16·45	0·857	0·335	22·500	0·197	Jaquet.[18]

[1] *Med. Chem. Untersuch.*, Berlin, 1868, Heft 3, S. 370.

[2] The results of the analyses made by Dr. Kossel were published in a paper by Hoppe-Seyler, entitled "Weitere Mittheilungen ueber die Eigenschaften des Blutfarbstoffs—Das Oxyhämoglobin des Pferdeblutes," *Ztschr. f. physiol. Chem.*, Strassburg, 1878–79, Bd. ii. S. 149–155.

[3] "Ueber das Oxyhämoglobin des Schweines," *ibid.*, 1882–83, Bd. vii. S. 57–68.

[4] "Ueber die Grösse des Hämoglobinmolecüls," *ibid.*, 1886, Bd. x. S. 16–34.

[5] "Ueber das Oxyhämoglobin des Pferdes," *Ztschr. f. physiol. Chem.*, Strassburg, 1883–84, Bd. viii. S. 358–365; "Beiträge zur Lehre vom Blutfarbstoffe," in *Beitr. z. Physiol. C. Ludwig z. s. Geburtst. etc.*, Leipzig, 1887, S. 74–81; "Neue Versuche zur Bestimmung des Sauerstoffscapacität des Blutfarbstoffs," *Arch. f. Physiol.*, Leipzig, 1894, S. 130–176. See especially S. 174–176.

[6] "Elementaranalyse des Hundeblut-Hämoglobins," *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 285–288; Beiträge zur Kenntniss des Blutfarbstoffes," *ibid.*, 1890, Bd. xiv. S. 289–296.

[7] This analysis, which is adduced for purposes of comparison, does not fall within the dates given above, having been published in 1868.

[8] *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 285.

[9] *Ibid.*, 1890, Bd. xiv. S. 289.

[10] *Ibid.*, 1878–79, Bd. ii. S. 149. Refer to Note 2.

[11] *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxi. S. 240.

[12] Hüfner, *Ztschr. f. physiol. Chem.*, Strassburg, 1883–84, Bd. viii. S. 358. This paper contains the results of Bücheler's researches, which have been carried out under Hüfner's direction, and had appeared as a Tübingen Dissertation in 1883.

[13] *Ibid.*, Strassburg, 1886, Bd. x. S. 16.

[14] *Beitr. z. Physiol. C. Ludwig z. s. Geburtst. etc.*, Leipzig, 1887, S. 74.

[15] *Arch. f. Physiol.*, Leipzig, 1894, S. 174.

[16] *Ztschr. f. physiol. Chem.*, Strassburg, 1882–83, Bd. vii. S. 57.

[17] *Beitr. z. Physiol. C. Ludwig z. s. Geburtst. etc.*, Leipzig, 1887, S. 74.

[18] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiv. S. 289.

Were we to admit the accuracy of the work of all the observers, whose results are exhibited on the table on p. 199, we should be forced to the conclusion that hæmoglobin is a body which does not only vary considerably in composition in different animals, but does not possess a constant composition even in different individuals of the same species. Thus, whilst Kossel found the percentage of carbon in the oxyhæmoglobin of the horse to be 54·87, and the mean of a large number of analyses by Kossel, Otto, and Bücheler gave 54·68, Zinoffsky, as a result of his analyses (only two in number, so far as the carbon and hydrogen are concerned!), found the percentage of carbon in the hæmoglobin of the horse to be 51·15 (!!). A body in which the carbon differs by 3·72 per cent. in different specimens cannot, it will be argued, be a chemical individual. But to draw this conclusion in reference to hæmoglobin from the facts in our possession would certainly be an error. The discrepancies between the results of the analyses of the hæmoglobin of the same animals are doubtless due to differences in the purity of the substance analysed, and to errors of analysis. The preparation of perfectly pure oxyhæmoglobin, entirely free from contamination with other constituents of the blood corpuscles and from products of decomposition, is much more difficult than has, until very recently, been supposed. In the attempt to purify the substance by crystallising it as frequently as practicable, nearly all observers have in all probability decomposed it, and have afterwards analysed a mixture of oxyhæmoglobin and products of its decomposition. How far this is the source of the above discrepancies must now, in the light of recent spectrophotometric work, be carefully enquired into. Moreover, assuming that perfectly pure crystallised oxyhæmoglobin is at the disposal of the analyst, the task of drying without decomposing it is one of peculiar difficulty, concerning the method of execution of which the chemists who have carried out the researches under discussion have been by no means agreed. Thus, whilst some (following Hoppe-Seyler's directions) have dried the oxyhæmoglobin intended for analysis, in the first instance *in vacuo* at 0° C., and only afterwards at higher temperatures, others (Zinoffsky, Hüfner, Jaquet) have dried the substance *in vacuo* at ordinary temperatures (15° to 18° C.), and subsequently at 110° to 115° C.

It is conceivable, nay probable, that some of the differences in the results of different observers may have depended upon the above-mentioned difference in the treatment of the substance analysed. But, unquestionably, some of the best marked differences must depend upon differences in the method of analysis employed (*e.g.* where one observer determines the N in oxyhæmoglobin by Will and Varrentrapp's method, whilst another employs Dumas' method), and upon accidental errors of analysis, which can easily be rendered obvious, by making a considerable number of analyses.

For instance, it appears to me that the percentage of carbon given by Zinoffsky,[1] as representing the proportion of this element in the hæmoglobin of the horse, *must* be due to imperfect combustion. Whilst this observer carried out the determinations of iron and sulphur in the hæmoglobin of the horse in the most elaborate and perfect manner, making many analyses of each of three separately prepared specimens of crystallised hæmoglobin, he rested satisfied with only two determinations of carbon and hydrogen, and two determinations of nitrogen (the latter by the method of Will and

[1] "Ueber die Grösse des Hämoglobinmolecüls," *Ztschr. f. physiol. Chem.*, Strassburg 1886, Bd. x.

Varrentrapp), though the results which he obtained differed in a remarkable manner from all those of previous observers. It is clear that whilst very great value must be attached to the determination of the iron and sulphur contained in hæmoglobin, made by Zinoffsky, his conclusions as to the percentage of carbon and hydrogen must be rejected, as being based upon an insufficient number of analyses, and as being in all probability incorrect. This opinion is supported by the remarkable discrepancy between his results and those of other observers —a discrepancy which cannot be accounted for by differences in purity of the bodies analysed.

While it is almost inconceivable, and against the weight of evidence, that hæmoglobin derived from animals of the same species should not have a constant composition, the differences in centesimal composition which certainly do exist between the hæmoglobin of certain animals and that of others cannot surprise us when we reflect that hæmoglobin does exhibit marked physical differences in different animals — that it exhibits variations in crystalline form, in the amount of water of crystallisation, and in solubility.

The study of the general results of the ultimate analyses of oxyhæmoglobin made of recent years forces us assuredly to the conclusion that new and still more precise investigations are needed before we can lay claim even to so limited a knowledge as that of its *precise* centesimal composition. Nevertheless, it would be wrong to leave the study of the more recent researches without drawing attention to certain of the numerical results obtained, which are more deserving of confidence than others.

The most characteristic and the most important of the elements which enter into the composition of hæmoglobin is its iron. Iron is the typical element in a molecular group which exists and possesses identical chemical and physical properties in all the varieties of hæmoglobin with which we are acquainted. Besides furnishing us with data by which the molecular weight of hæmoglobin may be calculated, the amount of iron appears to bear a definite relation to the quantity of the dissociable oxygen and carbonic oxide which hæmoglobin combines with. For these reasons, an extremely accurate determination of the iron in hæmoglobin, carried out with all the precision which the present state of science permits of, has been a great desideratum. Such determinations have been carried out by Zinoffsky, Jaquet, and Hüfner (see p. 199).

Hæmoglobin of	Fe per cent.	Authority.
Dog . .	0·336	Jaquet.
Horse . .	0·335	Zinoffsky.
Ox . . .	0·336	Hüfner.
Hen . .	0·336	Jaquet.

These observers have determined the proportion of iron in the oxyhæmoglobin of the dog, the horse, the ox, the pig, and the hen. They have shown: First, that the amount of iron in the blood-colouring matter of these animals is decidedly smaller than had been assumed on the basis of the older analyses. Secondly, that in the animals mentioned the percentage of iron in the hæmoglobin is identical, so that we may conclude that in these very different animals, in spite of

the discrepancies between the results of the ultimate organic analyses yet made, the oxyhæmoglobin possesses the same molecular weight. The concordance between the more recent determinations of the iron of oxyhæmoglobin is well shown in the table given on the previous page.

On the assumption that one molecule of hæmoglobin contains one atom of iron, the molecular weight of the hæmoglobin of the dog, horse, ox, and hen would be 16,669, and this result is borne out, as will be afterwards shown, by the volume of oxygen or of carbonic oxide which enters into combination with the blood-colouring matter.

In addition to the estimation of the iron in hæmoglobin, that of the sulphur has been carried out with remarkable care by Hüfner,[1] Zinoffsky, and Jaquet; and their results, whilst establishing that the centesimal composition of the blood-colouring matter of all animals is not identical, show that in hæmoglobin the sulphur stands to the iron in definite relations.

Thus Zinoffsky's analyses appear to establish that in the hæmoglobin of the horse the sulphur is to the iron in the relation of two atoms of the former to one of the latter element, and Hüfner has shown that exactly the same relation obtains in the case of the hæmoglobin of the ox and the pig. On the other hand, Jaquet's analyses of the hæmoglobin of the dog indicate that in it three atoms of sulphur correspond to one atom of iron. When, in a subsequent section, we shall examine the products of decomposition of hæmoglobin, we shall show that, under the influence of acids and alkalies, the blood-colouring matter breaks up into an iron-containing body (of which the composition and the properties vary according to the presence or absence of oxygen during the decomposition) and into an albuminous body or bodies. The sulphur of hæmoglobin belongs to the albuminous part of the molecule, and the difference in the relation of S to Fe, brought out by the researches of Hüfner, Zinoffsky, and Jaquet indicates that the albuminous moiety of the hæmoglobin molecule varies in different animals, and that among the points of difference is the difference in the proportion of sulphur. This point will be certainly cleared up by future researches specially directed to its elucidation; it may be remarked, however, that the proportion of sulphur in different albuminous bodies does exhibit great variations.

It appears to me, moreover, that we must not lose sight of the possibility (even when there is no evidence afforded by ultimate organic analysis of there being a difference in the percentage composition of the albuminous part of the hæmoglobin moiety), and indeed probability, *that hæmoglobins varying in certain physical properties may be formed by the linking of the iron-containing molecule to various polymeric combinations of the same albuminous molecule.*

Although it is highly probable that the molecular weight of the hæmoglobin of the dog and of the ox (16,669), as determined by the iron determinations of Jaquet and Hüfner, and by determinations by Hüfner of the volumes of O and CO with which hæmoglobin combines, has been ascertained with correctness, or nearly so, the discrepancies in the results of the determinations of C, H, and N, made by different observers, are too great to warrant our placing confidence in the empirical formulæ which have been assigned to hæmoglobin. Of these

[1] "Bestimmung d. Sauerstoffscapacität d. Blutfarbstoffs," S. 76.

empirical formulæ, that calculated by Jaquet for the hæmoglobin of the dog is probably the nearest the truth, namely—

$$C_{758}H_{1203}N_{195}S_3FeO_{218}$$ [1]

Why should hæmoglobin possess so enormously high a molecular weight? The question suggested itself to the acute mind of Bunge, who has furnished us with one reason which is eminently suggestive: "The enormous size of the hæmoglobin molecule," says this writer, "finds a teleological explanation, if we consider that iron is eight times as heavy as water. A compound of iron, which would float easily along with the blood current through the vessels, could only be secured by the iron being taken up by so large an organic molecule." [2]

When discussing the compounds and products of decomposition of oxyhæmoglobin and hæmoglobin, we shall have again to revert to and further examine certain of the facts which have found a place in this section.

The crystalline form, the amount of water of crystallisation, the solubility, and the diffusibility of oxyhæmoglobin.—Although, as has already been stated, the oxyhæmoglobin of different animals varies considerably in the facility with which it crystallises, we now know that the hæmoglobin of all animals, without exception, may, by suitable treatment, be obtained in the crystalline form.[3] Great differences exist in the solubility of the blood-colouring matter obtained from different animals, and, as might have been anticipated, the blood of these animals whose hæmoglobin is least soluble (as the rat, the guinea-pig, and the squirrel) yields crystals of oxyhæmoglobin most readily; whilst the converse is also true, *i.e.* the oxyhæmoglobin of man, of the rabbit, the sheep, and the ox, all of which are exceedingly soluble, yield crystals with considerable difficulty. It was, indeed, long supposed to be impossible to obtain large quantities of oxyhæmoglobin from the blood of certain of these animals.

As a rule, crystals of oxyhæmoglobin are of such a size that their form, and even their crystalline nature, cannot be made out by the naked eye. The blood of certain animals, however, as the dog, and particularly the horse, yields under favourable circumstances rhombic prisms of macroscopic size. From horse's blood Hoppe-Seyler frequently obtained prisms over 5 mm. in length and 4 mm. in thickness. The colour of crystals of oxyhæmoglobin appears different, according to their size or the number aggregated together.[4] Thus the finest needles or prisms of oxyhæmoglobin, when seen singly under the microscope, appear almost colourless, or possess the yellowish tint characteristic of the coloured corpuscles. On the other hand, large crystals, or consider-

[1] From the results of Hüfner's analyses of the hæmoglobin of the ox, but substituting his most recent determinations (1894) of the iron for the older ones, published in 1887, I have calculated for the hæmoglobin of this animal the formula—

$$C_{759}H_{1208}N_{210}S_2FeO_{204}$$

[2] G. Bunge, "Text-Book of Physiological and Pathological Chemistry." Translated by L. G. Wooldridge: London, 1890, p. 24.

[3] It was Dr. Otto Funke who first asserted, as the result of his own researches, "that all blood is capable of crystallisation, whatever animal or organ it may be taken from."—"Explanation of the Plates" of his "Atlas of Physiological Chemistry," p. 15 (see p. 205, note 1).

[4] F. Hoppe-Seyler, "Das Oxyhämoglobin des Pferdeblutes," *Ztschr. f. physiol. Chem.*, Strassburg, 1878–79, Bd. ii. S. 149.

able aggregations of the smaller crystals, exhibit, like aggregations of blood corpuscles, the red colour characteristic of the blood.

We shall now examine successively the most important facts connected with the (1) form, (2) quantity of water of crystallisation, (3) solubility, presented by crystallised oxyhæmoglobin, (4) diffusibility.

1. *Form.*—(*a*) The blood of man and of the immense majority of animals yields oxyhæmoglobin which crystallises in rhombic prisms or needles belonging to the rhombic system.

(*b*) The oxyhæmoglobin of the guinea-pig presents crystals which were described by Lehmann as regular octohedra. They were, however, shown by the eminent crystallographer v. Lang[1] to be tetrahedra belonging to the rhombic system.

The blood of certain birds,[2] and occasionally apparently of the rat[3], [4], [5], yields crystals of the same form.

(*c*) The oxyhæmoglobin of the squirrel crystallises normally in the form of six-sided plates belonging, as proved by v. Lang, to the hexagonal system. These crystals had been first described by Lehmann and Kunde. The blood of the hamster (*Cricetus vulgaris*) contains oxyhæmoglobin which crystallises, as Lehmann showed, in rhombohedra and six-sided plates belonging to the hexagonal system. Halliburton,[6] who has studied the crystallography of oxyhæmoglobin with great care, has made the interesting observation that "after recrystallising squirrel's hæmoglobin several times the hexagonal constitution of the crystals is broken down, and the crystals obtained are either rhombic prisms or a mixture of these with rhombic tetrahedra."

Rollett,[7] taking for granted that oxyhæmoglobin, from whatever source obtained, possessed the same chemical composition, argued, from the fact of its crystallising generally in the rhombic, but in the case of the squirrel in the hexagonal system, that oxyhæmoglobin should be looked upon as dimorphous.

Halliburton, however, with perfect correctness, hesitates to admit this view, which could only be held if we were certain that the hæmoglobins whose crystals belong to different systems possess identical composition, and suggests that perhaps the difference in the crystalline form, as well as the difference in solubility of the hæmoglobins which crystallise differently, depends upon varying quantities of water of crystallisation—that, in fact, the hæmoglobins which crystallise in different systems represent "different hydrates of oxyhæmoglobin."[8] This may be the case, though it appears to me that the cause of the difference lies deeper.

It has been previously stated—and the grounds for the statement will be given in a subsequent section—that, notwithstanding the perplexingly discordant results of the analyses of oxyhæmoglobin, there is, in the hæmoglobin of all animals, absolute identity of the essential iron-containing nucleus, *i.e.*

[1] In a paper by A. Rollett, entitled "Versuche u. Beobachtungen am Blut, nebst krystallographisch. u. optisch. Mittheilungen ueber die Blutkrystalle von v. Lang," *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlvi. S. 66–98.

[2] Halliburton, "Text Book of Chemical Physiology," London, 1891, S. 270.

[3], [4] Kunde, Lehmann, see Preyer, "Die Blutkrystalle," S. 38.

[5] Hoppe-Seyler, "Ueber die Krystallformen der Blutkrystalle," *Med. Chem. Untersuch.*, Berlin, 1868, S. 195.

[6] "Preliminary Communication on the Hæmoglobin Crystals of Rodents," *Journ. Physiol.*, Cambridge and London, 1886, vol. vii. p. 2; *Quart. Journ. Micr. Sc.*, London, vol. xxviii. p. 181.

[7] *Loc cit.*

[8] Halliburton, "Text Book of Chemical Physiology," section on the "Crystallography of Oxyhæmoglobin," pp. 270–274. The student is recommended to read this interesting and suggestive section.

of that moiety of the molecule on which its colour and its physiological function depends. At the same time, there is such a difference in the ratio of S:Fe in the hæmoglobin of certain animals as renders it highly probable, or rather certain, that, in the hæmoglobin of different animal groups, the albuminous moiety of the complex molecule differs. Such being the case, it is not surprising that certain of the physical characters of hæmoglobin, such as crystalline form and solubility, should exhibit variations.[1] Nor can we lose sight of the possibility, to which I have already drawn attention, that the differences in the hæmoglobins of certain animals may be due to their being formed by the linking of the iron-containing molecule with different polymers of the same albuminous group. The existence of hæmoglobins varying somewhat in their percentage of iron renders this view highly probable.

2. *Quantity of water of crystallisation.* — Remarkable difficulties encounter the observer in his attempts to determine the amount of water of crystallisation of oxyhæmoglobin, and considerable discrepancies are to be noticed in the results obtained by different processes.

In order to make the determination, pure oxyhæmoglobin is dried *in vacuo* at 0° C., and after ceasing to lose weight under these conditions it is heated to a temperature of 115° C.

The following are some of the principal and most reliable results obtained:—

Oxyhæmoglobin.	Water of Crystallisation per cent.	Authority.
Dog	3·4	Hoppe-Seyler.
Horse	3·94	Hüfner.
Pig	5·9	Otto.
Guinea-pig . . .	6	Hoppe-Seyler.
Squirrel	9	Hoppe-Seyler.

According to Bohr,[2] the water of crystallisation of oxyhæmoglobin may vary in amount between 1·2 and 6·3 per cent., but these results, like others obtained by the same author, and to which reference has been made (see p. 192), are explicable by the fact that his preparations of hæmoglobin did not represent the pure substance, and contained products of decomposition.

Without taking Bohr's results into consideration, there can be no doubt that crystals of oxyhæmoglobin of different animals exhibit differences in the amount of water of crystallisation. Assuming the above results to be correct, the highly soluble oxyhæmoglobin of the pig, which crystallises in rhombic prisms, possesses the same amount of water of crystallisation as the very sparingly soluble oxyhæmoglobin of the guinea-pig, separating in the form of tetrahedra.

3. *Solubility.* — The difficulties which encounter the observer in

[1] The reader is referred to an admirable account of all the researches on the Crystallography of Hæmoglobin, up to the date of its publication (1871), to the chapter entitled "Krystallformen des Blutroths," in Preyer's work, "Die Blutkrystalle." Very fine coloured engravings of the hæmoglobin crystals of various animals—amongst others, of man, the guinea-pig, and the squirrel—are to be seen in Funke's "Atlas of Physiological Chemistry," being a Supplement to Lehmann's "Physiological Chemistry," London, printed for the Cavendish Society, 1853. See plate x. and pp. 15–17 of the appended letterpress.

[2] "Exp. Untersuchungen ü. die Sauerstoffaufnahme des Blutfarbstoffes," Copenhagen, 1885.

determining the water of crystallisation of the blood-colouring matter are surpassed by those attending the estimation of its solubility. It is doubtless in some measure due to the difficulty, almost the impossibility, of eliminating every trace of certain of the reagents (especially the alcohol), employed in the preparation of the body, that any attempts to determine with precision the solubility of oxyhæmoglobin have failed. The chief cause of the discrepancies between the observations of different observers is, however, probably that they were unaware of the physical, and perhaps also chemical, changes which hæmoglobin undergoes in the process of recrystallisation.

The oxyhæmoglobin of all birds, of the ox, of the pig, and of man is distinguished by its great solubility, the relative solubility increasing in the above order. Next in order of solubility comes the hæmoglobin of the horse, dog, squirrel, guinea-pig, and rat, the latter being certainly the least soluble.

According to C. Schmidt, 100 grms. of water at 18° C. dissolve 15·59 grms. of the crystallised oxyhæmoglobin of the dog. From the fact that the oxyhæmoglobin analysed by C. Schmidt when ignited yielded on an average 0·91 per cent. of P_2O_5, we are in a position to state that the body he experimented with was very impure, and consequently that his estimate of its solubility in water possesses no value. Hoppe-Seyler found that 100 c.c. of water at 5° C. dissolved 2 grms. of the dry oxyhæmoglobin of the dog.

Lehmann found that one part of the dry crystallised oxyhæmoglobin of the guinea-pig required 597 parts of water to dissolve it, but the temperature at which the determination was made is not stated;[1] moreover, it is more than doubtful whether the substance experimented with was pure.

The present state of our knowledge permits us, therefore, to state that the oxyhæmoglobin of different animals differs in no property so remarkably as in its solubility in water. It appears, further, that oxyhæmoglobin—which, according to the more recent researches, contains the same percentage of iron (that of the horse, ox, dog, and pig), and therefore presumably possesses the same molecular weight, and which, further, crystallises in the same manner—exhibits marked differences in solubility. As the oxyhæmoglobins of the horse and of the dog seem, in so far as the water of crystallisation is concerned, to be identical, and as the researches of Hüfner and his school have proved the identity of the iron-containing part of the molecule in the hæmoglobin from the most different animals, we are, it appears to me, driven to the conclusion that the difference in solubility *must be due* to differences in the albuminous residue in the hæmoglobin molecule.

Solubility in liquids other than water.—Oxyhæmoglobin is soluble in highly diluted solutions of ammonia, and the other caustic alkalies, and their carbonates. These solutions resist decomposition much longer than aqueous solutions of hæmoglobin.[2] Kühne states that a highly dilute ammoniacal solution of oxyhæmoglobin will remain in great part unchanged for several weeks at ordinary temperatures. Stronger solutions of the caustic alkalies or their carbonates induce decom-

[1] W. Preyer, "Die Blutkrystalle," S. 55.

[2] Based upon these facts is the method, introduced by Hüfner, of diluting blood or solutions of oxyhæmoglobin with solutions containing 0·1 per cent. of NaOH. Such solutions are much more transparent than purely aqueous solutions, and are therefore most valuable for the purposes of spectroscopic researches.

position of oxyhæmoglobin with a rapidity which depends upon their concentration.

Oxyhæmoglobin is soluble in highly diluted alcohol, the solutions resisting putrefaction much longer than aqueous solutions. By contact with even highly dilute alcohol, crystals of oxyhæmoglobin become much more sparingly soluble in water. Oxyhæmoglobin is insoluble in absolute alcohol. When crystallised oxyhæmoglobin is treated with a large excess of absolute alcohol, it is under favourable circumstances converted into an insoluble crystalline modification, to which Nencki and Sieber have given the name of *parahæmoglobin*.[1] This body cannot be looked upon as a chemical individual. Oxyhæmoglobin is insoluble in chloroform, benzol, and carbon disulphide.

4. *Diffusibility.*—Oxyhæmoglobin offers a remarkable example of a soluble crystalline body, which, judged by its power to pass through a septum of parchment paper, must be declared to be absolutely non-diffusible. This character depends upon the enormous size of its molecule.

Comparison of the action of certain reagents on solutions of oxyhæmoglobin and on solutions of albuminous bodies.—It has already been incidentally stated that in hæmoglobin an iron-containing body is linked to an albuminous body or bodies, and reference has been made to the fact that, under the action of various agents, oxyhæmoglobin breaks up into the iron-containing hæmatin, and into albuminous bodies. Although the decomposition of hæmoglobin and its products will be considered in some detail in a future section, it is convenient in this place to refer to this point, and to state that when oxyhæmoglobin is decomposed so as to yield hæmatin and albuminous substances, the former amounts approximately to 4 per cent. and the latter to 96 per cent. of the original hæmoglobin.

Such being the case, it is of particular interest to contrast the action of certain reagents on solutions of albuminous bodies, and on solutions of oxyhæmoglobin.

Solutions of oxyhæmoglobin differ remarkably from solutions of albuminous bodies in their behaviour towards a large number of reagents.

As Kühne pointed out long ago,[2] all those tests for albumin which do not immediately bring about a decomposition of oxyhæmoglobin, furnish a negative result when applied to aqueous solutions of this body. Cupric and ferrous sulphates, mercuric chloride, silver nitrate, neutral and basic acetates of lead, all of which precipitate albuminous solutions, occasion (so long as the body remains undecomposed) no precipitate—not even cloudiness—when added to solution of oxyhæmoglobin. So soon, however, as the red colour of oxyhæmoglobin has disappeared under the action of any one of the above salts, and the brown colour due to hæmatin has appeared (a result which they all sooner or later bring about), the characteristic albuminous precipitates appear.

[1] M. Nencki und N. Sieber, "Untersuch. ueber die Blutfarbstoff," *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1885, Bd. xviii. S. 392 ; M. Nencki und B. Lachowitz, "Ueber das Parahämoglobin," *ibid.*, Bd. xviii. S. 2126. The reader is referred for a criticism of Nencki and Sieber's researches on parahæmoglobin, to a paper by Hoppe-Seyler, entitled "Ueber Blutfarbstoffe und ihre Zersetzungsproducte," *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 531.

[2] "Lehrbuch der physiolog. Chemie," Leipzig, 1866, S. 207.

Other reagents which bring about an instant decomposition of oxyhæmoglobin, and, consequently, instantly set free the albuminous matter, exhibit also, as might have been anticipated, the characteristic albumin reactions, *i.e.* behave towards a solution of hæmoglobin as if it were a solution of a native albumin. This remark applies to acetic acid and potassium ferrocyanide, to mercuric nitrate, to the concentrated mineral acids—reagents, all of which precipitate a solution of oxyhæmoglobin as they do solutions containing albuminous bodies.

When subjected to the action of heat, solutions of oxyhæmoglobin coagulate like solutions of the native albumins; but, doubtless, before the temperature of coagulation (64° to 68°·5 C.) is reached, the complex hæmoglobin molecule has already been decomposed—a supposition which is suggested by the following observation:[1]—If to an aqueous solution of crystallised oxyhæmoglobin of the dog a small quantity of sodium carbonate be added, on applying heat no coagulation occurs, even though the temperature be raised to 100° C. When, however, the temperature reaches 54° C., the colour of the solution instantly changes to deep brown, and spectroscopic examination indicates that the spectrum of oxyhæmoglobin has been replaced by that of alkaline hæmatin.

The Absorption of Light by Oxyhæmoglobin.

(*a*) **The visible spectrum.—Historical notes.**—The researches of Brewster and Herschel had shown that absorption-bands occur in the spectrum of light which has been passed through certain coloured gases, vapours, and coloured solutions, and the so-called *absorption spectra* of indigo and chlorophyll had been described before the time when Hoppe[2] made the discovery of the beautiful absorption spectrum of blood, distinguished by two very characteristic absorption-bands, situated in the region which intervenes between the lines of Frauenhofer, D and E.

This discovery at once enabled Hoppe to affirm that hæmatin, which had up to that time been generally looked upon as the true blood-colouring matter, does not exist as such in the blood corpuscles, but that it is a product of the decomposition of the colouring matter; that the latter, to which he afterwards gave the name of hæmoglobin, and which he recognised as forming the so-called blood crystals described by Kunde, Lehmann, and Funke, is the cause of the absorption-bands which he had discovered in the spectrum of diluted blood, and that this colouring matter, under the influence of heat, acids, and various other chemical agents, splits up into hæmatin and an albuminous substance or substances.

There can be no question that, although Hoppe, in a certain measure, appreciated the immense value of the knowledge which he had gained by his study of the optical properties of the blood, the full light which it was destined to shed on the function of the blood-colouring matter was only recognised when Professor Stokes, two years later, published his paper "On the Reduction and Oxidation of the Colouring Matter of the Blood."[3] The new facts acquired by the combination of chemical and optical methods in this research, and which at once shed a flood of light on phenomena which had until then been shrouded in darkness, enlisted as workers in this field

[1] Preyer, "Die Blutkrystalle," S. 61.

[2] Hoppe only assumed the name of Hoppe-Seyler in 1864. The paper containing his first observations on the spectrum of the blood bore the following title:—Professor Hoppe in Tübingen, "Ueber das Verhalten des Blutfarbstoffes im Spectrum des Sonnenlichtes," *Virchow's Archiv*, 1862, Bd. xxiii. S. 446–449.

[3] *Proc. Roy. Soc. London*, 1864, vol. xiii. p. 357.

many persons of distinction in all countries, amongst the first and most successful of whom were W. Preyer[1] in Germany, and Sorby and Ray Lankester in England. Amongst all, however, who by their work have contributed to the spectroscopic investigation of the blood, two appear to me to stand out pre-eminently—these are Vierordt and Hüfner. By the discovery of the first practical method of determining the *extinction-coefficient* of coloured liquids, and his elaboration of a general method for the quantitative analysis of colouring matters, a method capable of surprising refinement and accuracy, and which is based upon the relation which exists between the extinction-coefficient and concentration, Vierordt has placed both the sciences of physics and physiology under a lasting obligation.[2] To Hüfner belongs the merit of having developed and perfected the methods of spectrophotometry, but especially of employing it so as to obtain results of paramount importance to physiology, and which would have been unattainable without its aid. Not only has he, by his own long-continued researches, and those of his pupils, determined the spectrophotometric constants of hæmoglobin and its compounds with oxygen and carbonic oxide, but he has by spectrophotometry succeeded in determining the absolute and relative amounts of reduced and oxyhæmoglobin existing side by side in the blood. He has further shown that, as we now know the volume of oxygen which can combine with 1 grm. of hæmoglobin, by determining the amount of hæmoglobin and of oxyhæmoglobin coexisting in any specimen of blood, we possess data enabling us to calculate the volume of the dissociable or respiratory oxygen of the blood, without having recourse to direct determinations by means of the mercurial pump and gas analysis.

Further, by the method of spectrophotometry, combined with the results of chemical investigation, Hüfner has furnished us with the proof that, in spite of the differences in many physical characters, and even in centesimal composition presented by the blood-colouring matter of different animals, the coloured iron-containing group existing in hæmoglobin, upon which its essential physiological functions depend, is identical in all.[3]

General description of the visible spectrum of oxyhæmoglobin.—*Instruments required.*—For the study of the visible, as distinguished from the photographic spectrum of the blood, or of oxyhæmoglobin, the spectroscopes which are in common use in physical and chemical laboratories may be employed, providing the dispersion of their prisms be not too great. A spectroscope of the ordinary Bunsen type, provided with a single good flint-glass prism, is infinitely to be preferred for the study of absorption spectra to an instrument with two prisms, for, with the greater dispersion, absorption-bands appear much less clearly defined than with the smaller. Direct vision spectroscopes of the Browning or Hofmann patterns, or microspectroscopes, *i.e.* direct vision spectroscopes adapted to the eyepiece of the compound microscope, may be employed; and the second class of these instruments renders great services in the investigation of minute quantities of colouring matters—as, for instance, in the examination of the optical characters of the colouring matters of the tissues.

It is advisable, indeed for the purposes of original research indispensable, that the spectroscope employed should furnish means of determining accur-

[1] Preyer's monograph, entitled "Die Blutkrystalle," which appeared in Jena in 1871, still continues indispensable to the physiological chemist. It is replete with original observations of great value, and establishes that Preyer had no unimportant share in the development of our knowledge of the blood-colouring matter.

[2] Karl Vierordt, "Die Anwendung des Spektral-apparates zur Photometrie der Absorptionsspektren und zur quantitativen chemischen Analyse," Tübingen, 1873; "Die quantitative Spektralanalyse in ihrer Anwendung auf Physiologie, Physik, Chemie, und Technologie," Tübingen, 1876.

[3] As the chief of Hüfner's papers have been already quoted, or will be referred to subsequently in detail, their dates and titles are not given in this place.

ately the position of any line or the boundaries of any absorption-band observed in the spectrum, it being usual to express the position in terms of the wave length of the light corresponding to it. With this object the spectrum of sunlight is observed, and the position of the principal lines of Frauenhofer is determined in reference to the divisions of the photographic scale, or, in the case of the finer spectroscopes and spectrometers, in reference to the divisions of the graduated circle of the instrument. From the results of these observations a curve is readily plotted, enabling the experimenter at any time to convert the readings of the arbitrary scale of his instrument into wave lengths.[1]

For all exact spectroscopic work the eyepiece of the spectroscope should be provided with cross-threads; and, when employed in the investigation of absorption spectra, if possible with the arrangement employed in spectrophotometry, which enables the observer to limit, by a variable slit in the eyepiece, any particular spectral region and to shut out of the field of view the remainder of the spectrum.

As a source of light, for some investigations the light of the sun reflected from the mirror of a heliostat driven by clock-work is desirable; for general purposes the light of the sun, reflected from a white surface, may be employed. Artificial sources of illumination possess the great advantage of being available at all times, and susceptible of considerable constancy. A gas lamp, furnished with the Auer incandescent burner, is the best of all lamps for the examination of absorption spectra.

In examining the absorption-spectra of liquids, it is convenient to employ cells or troughs with perfectly parallel glass or quartz sides, which are a definite width apart. Such vessels are made according to the model of Hoppe-Seyler, and sold under the name of *hæmatinometers* (Fig. 23), the internal surface of the parallel glass plates being exactly 1 cm. apart, and the little trough being so arranged as to be readily taken to pieces for cleaning. The small troughs employed in spectrophotometry, and which are usually constructed with great care, are well adapted to the general purposes of the spectroscopist.

Instead of a vessel of which the sides are at a constant and known distance apart, it is convenient for many purposes to employ the so-called *hæmatoscope*, or *hæmoscope*, of Hermann,[2] as shown in the accompanying woodcut (see Fig. 24). F is a glass plate, forming the anterior wall of the tube D, which is supported on the stand A. C is a metallic tube, sliding in and out of the tube D, and closed anteriorly by a glass plate parallel to F. E is a funnel communicating with the interior of D F B. By sliding the piston C in and out of the tube D, the capacity of the vessel D F B and the depth of a stratum of liquid contained between the two glass plates, may be modified at will within wide limits.

The depth of the stratum is read off by the aid of a millimetre scale, engraved on the sliding tube C.

As the absorption of light passing through a coloured liquid depends upon the number of absorbing molecules in its path, by doubling the thickness of the stratum of a coloured liquid examined, we obtain the same result as by examining a solution of double concentration. With such a contrivance as the hæmatoscope, we are, within certain limits, able therefore to obtain the same result with a solution of constant concentration as with a large number of solutions of which the concentration varies in known proportions.

[1] In a work intended for the advanced student of physiology, it appears superfluous to enter into such details concerning the construction of the spectroscope, or the method of working with it, as can be learned in all courses of practical physics, or may be found in any elementary treatise devoted to this branch of science.

[2] "Notizen für Vorlesungs und andere Versuche," *Arch. f. d. ges. Physiol.*, Bonn, Bd. iv. S. 209.

The spectrum as seen with solutions of varying concentration.—When well-arterialised defibrinated blood (containing on an average from 12 to 14 per cent. of oxyhæmoglobin) is diluted with nine times its volume of distilled water, and a stratum 1 cm. thick is brought before the slit of the spectroscope, it will be found that the whole of the spectrum is absorbed, with the exception of the red end, or rather of those rays having a wave length greater than about 600 millionths of a millimetre (λ 600).

If, now, the blood solution be gradually diluted, a point is reached at which the spectrum is (proceeding from the red end) clear up to D (λ 598), and a strip of green is visible between *b* and F (λ 518·3–λ 486·1). Between D and *b* the absorption is intense (see Plate I., Spectrum 4), and beyond F no trace of light appears. On diluting still further, that

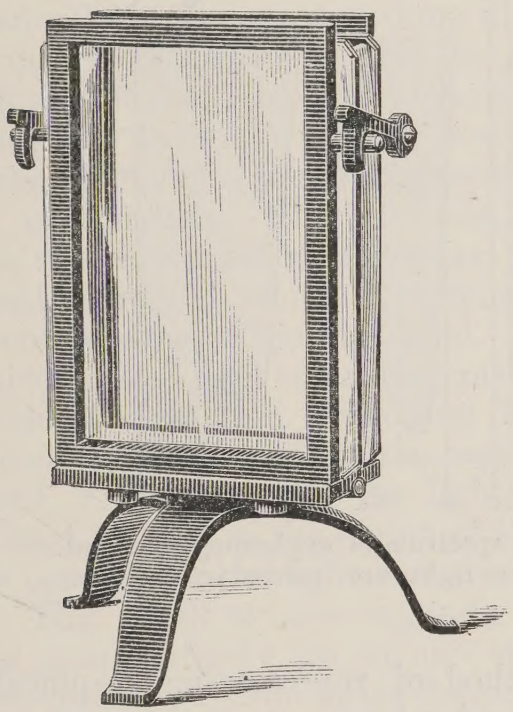

Fig. 23.—The hæmatinometer.

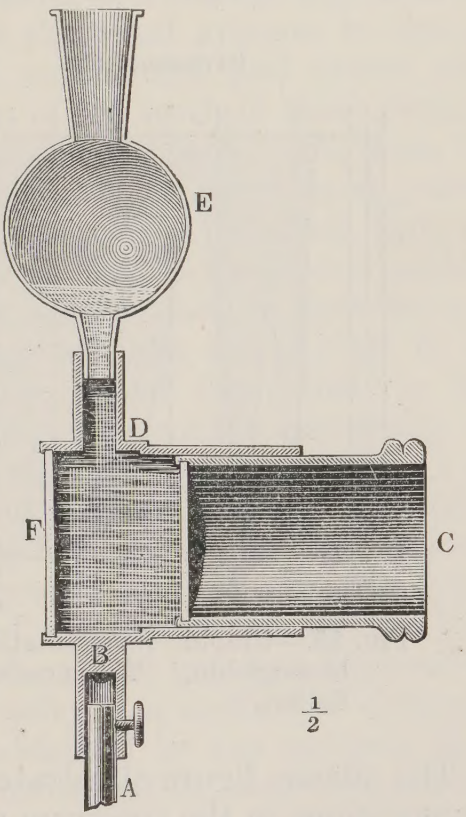

Fig. 24.—The hæmatoscope.

which appeared as a single wide absorption-band between D and *b*, and afterwards as the solution was progressively diluted between D and E, is seen to resolve itself into two distinct absorption-bands, separated by a green interspace; the violet end of the spectrum is still powerfully absorbed (Plate I., Spectrum 3).

Of the two absorption-bands just referred to, the one next to D is narrower than its fellow; it has more sharply defined borders, *and to the eye appears more intense*; its centre corresponds to λ 579, and we may conveniently distinguish it as the absorption-band α in the spectrum of oxyhæmoglobin.

The second of these absorption-bands, *i.e.* the one next to E, which we shall designate the band β, is broader, has less sharply-defined edges, and its centre corresponds approximately to λ 553·8. Between the two bands is a green interspace.

On diluting the solution more and more largely, and continuing to examine a stratum 1 cm. thick, the absorption of the violet end becomes

less and less, and the whole spectrum as far as G appears beautifully clear, except where the two absorption-bands are situated (Plate I., Spectrum 2). If dilution be pushed still further, these disappear; before they vanish they appear as faint shadows across the limited region which they occupy. The band α is said to disappear last. I find, however, that whenever I can detect α I am able to detect a faint shadow in the position of λ 540–λ 550. When the bands are *just* perceptible, there is no obvious absorption of either the red or the violet end of the spectrum.

The two absorption-bands of oxyhæmoglobin are seen in greatest perfection when a stratum 1 cm. thick of a solution containing 1 part per 1000 of oxyhæmoglobin is examined; this corresponds to a solution made by diluting from 1·2–1·4 parts of blood to 100. They are still perceptible when the solution contains 1 part oxyhæmoglobin in 100,000 parts of water (1 grm. in 10 litres).

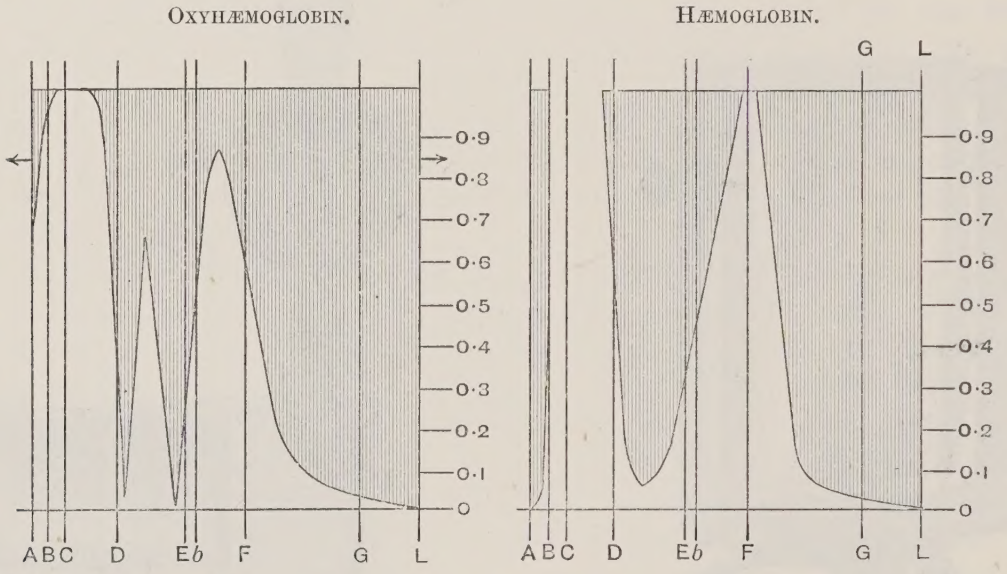

FIG. 25.—Graphic representation of the spectrum of oxyhæmoglobin and hæmoglobin. The numbers on the right are percentages.—After Rollett.

The above figure illustrates a method of representing graphically the variations in the spectrum of the blood-colouring matter, corresponding to all concentrations (a stratum of 1 c.c. being examined).[1]

In these diagrams the position of the principal Frauenhofer lines is shown; the numbers on the right indicate percentages of the blood-colouring matter. The shaded part of the diagram indicates absorption of light. By drawing lines parallel to the abscissæ we at once observe the character of the absorption spectrum which corresponds to the concentration indicated at the right-hand side of each diagram. Thus, by inspection of the left-hand diagram, we learn that solutions of oxyhæmoglobin, containing more than 0·65 per cent., exhibit a single broad absorption-band in the visible spectrum, owing to the fact that the two absorption-bands α and β have run together, and that the green interspace between b and F is shown only by solutions of less concentration than from 0·8 to 0·9 per cent. When the absorption of this part of the spectrum is complete, only orange and red remain unabsorbed.

By placing the solution of oxyhæmoglobin in a wedge-shaped cell,

[1] A. Rollett, "Physiologie des Blutes," Hermann's "Handbuch," Leipzig, 1880, Bd. iv. Th. 1, S. 48.

the slit being perpendicular to the edge of the wedge, the accuracy of the diagram can be realised objectively, each section of the slit forming a spectrum corresponding with a given thickness of stratum, which increases in a continuous manner from the edge towards the base of the wedge. This method of examination was first employed by J. H. Gladstone.[1]

The theory and methods of spectrophotometry.—The spectrophotometric constants of oxyhæmoglobin.—(a) The theory.—Interesting and attractive though it undoubtedly is, the examination of an absorption-spectrum, or the comparison of allied absorption-spectra, by the unaided sense of sight, may be singularly deceptive.

The impression which the unaided eye enables us to form of the boundaries, the breadth, the intensity of an absorption-band, or of the extent and depth of a less defined general absorption, is often very fallacious. When, for instance, the absorption of a definite region of the spectrum commences and ceases abruptly, the band appears to the eye more intense than when the absorption commences and ceases more gradually.[2] The most striking illustration of the truth of these remarks is indeed furnished by the two oxyhæmoglobin bands. The first, less refrangible band (α), has always been described as much more intense than the second, which is broader and less sharply defined, and unquestionably this is the impression which we form by ordinary methods of examination. Vierordt[3] has, however, shown that, in opposition to the visual impression, a greater percentage of light is absorbed in the spectral region which corresponds to the second band than in that corresponding to the first band. Measuring, spectrophotometrically, the percentage of light remaining unabsorbed, after traversing a stratum 1 cm. broad of a solution containing 1 per cent. of defibrinated mammalian blood, he found that in the region of the first, apparently more intense band, 87 per cent. of the light was absorbed and 13 per cent. transmitted; whilst in the region of the second, apparently less intense band, 90 per cent. of the light was absorbed, and only 10 per cent. transmitted.

This result at once suggests the necessity of a method of determining quantitatively the amount of light absorbed by any medium whose absorption-spectrum forms the subject of investigation, instead of trusting to our unaided sense of sight. When, however, we are made acquainted with the remarkable and far-reaching conclusions which can be legitimately drawn from an accurate determination of the percentage of light of a definite wave length, absorbed by colouring matters existing in solution, the beauty and the importance of the method of spectrophotometry become apparent. Until Vierordt's discovery, those coloured bodies whose visible spectrum presented no definite absorption-bands, were held to be beyond the scope of spectroscopic research. Now, however, we know that a photometric study of the spectrum affords us not only the means of identifying them, but supplies us with a method for the quantitative analysis of colouring matters, surpassing all others in accuracy, and permitting, in certain cases, of the accurate determination of data not to be ascertained in any other way.

[1] J. H. Gladstone, "On the Use of the Prism in Qualitative Analysis," *Journ. Chem. Soc.*, London, 1858, vol. x. p. 79.

[2] A. Rollett, "Physiologie des Blutes," Hermann's "Handbuch," Leipzig, 1880, Bd. iv. Th. 1, S. 50.

[3] "Die Anwendung des Spektral-apparates zur Photometrie der Absorptionsspektren," Tübingen, 1873.

1. *Relation between the concentration of a solution and the percentage of light absorbed by it.*—Before investigating the theory of the methods of spectrophotometry, to be subsequently described, it is essential to examine (1) the relation which exists between the power of light absorption exerted by a coloured liquid of constant composition and the thickness of the layer traversed; (2) to study the influence of concentration on the absorption of light by a stratum of a liquid holding a colouring matter in solution.

It was shown by Lambert that if light of intensity I, by transmission through one layer of an absorbing medium of thickness 1, has its intensity reduced to $I\frac{1}{n} = \frac{I}{n}$ by transmission through d such layers, the *final intensity* of the light, which we shall represent by I', will be reduced to $\frac{I}{n^x}$, *i.e.* $I' = \frac{I}{n^x}$. Beer showed that Lambert's law holds good, not only for transparent solid media, but also for liquids, *i.e.* that the *amount of light absorbed by a solution of a given colouring matter of constant concentration is dependent upon the thickness of the stratum.*[1] *This law is only true, however, in respect to monochromatic light.*

We must now examine the influence of the concentration of a liquid containing a colouring matter in solution upon the percentage of light which it absorbs and transmits, when the stratum examined remains of a constant width, 1. It has been experimentally proved that the absorption exerted by a stratum of a coloured solution of known width is equal to that exerted by a stratum twice as thick of a solution of half the concentration; *i.e. the absorption which light undergoes in passing through a stratum of coloured liquid of unit thickness increases proportionally to the concentration.*

2. *Definition of the "extinction-coefficient."*—In their photo-chemical researches, studying the comparative absorption of light by different gases, Bunsen and Roscoe[2] introduced the conception of, and defined, the so-called *extinction-coefficient.* They ascertained the relative thicknesses of the strata of various media required to reduce the intensity of light passed through them to one-tenth of its initial value, and defined the extinction-coefficient as *the reciprocal of the number expressing the width of the stratum of a given medium, required to reduce the intensity of light passed through it to one-tenth its initial value.*

For any given coloured medium, *e.g.* a solution of a colouring matter of a definite strength, there must be a definite thickness of layer which we shall call d, capable of reducing the intensity of light to one-tenth its original value. The reciprocal of d is $\frac{1}{d}$, and if by ε we represent the extinction-coefficient,

$$\varepsilon = \frac{1}{d}$$

As will be shown in the sequel, the method of spectrophotometry discovered by Vierordt rests upon the determination of this constant ε, for particular, very limited, regions of the spectrum. The practical difficulties of varying the thickness of the stratum of a coloured liquid, until

[1] When the thicknesses of various strata increase in arithmetical, the intensities of the light decrease in geometrical, ratio.

[2] *Ann. d. Chem.*, Leipzig, 1857, Bd. ci. S. 238.

the intensity of the light remaining unabsorbed is reduced precisely to one-tenth, would be extremely great. Fortunately, the coefficient of extinction can be determined in a manner presenting far smaller practical difficulties and admitting of great accuracy.

If, instead of varying the thickness of the stratum of the coloured solution until the initial intensity of the light entering it is reduced to one-tenth its value, we invariably examine in our photometric investigations a stratum of unit width (say 1 cm.), or a stratum of known width, and possess the means of estimating the proportion of light which remains unabsorbed, we possess data enabling us to calculate the extinction-coefficient.

It was previously shown that $I' = \frac{1}{n^x}$ and that $\varepsilon = \frac{1}{d}$, and when $x = d$, $I' = \frac{1}{10}$. Then $\log I' = -x \log n$, and $d \log n = 1 \therefore \varepsilon = \log n = -\frac{\log I'}{x}$;

so that, if the thickness of the stratum traversed by the light be known, and the intensity of the unabsorbed light I' ascertained, the coefficient ε can be calculated. But if x be of the constant value 1 (say 1 cm.), then $\varepsilon = -\log I'$; that is to say, *the extinction-coefficient is equal to the negative logarithm of the unabsorbed light.* Let us suppose that by passing through a stratum of coloured solution 1 cm. wide, the intensity of light has been reduced to two-thirds its original value, then

$$\varepsilon = -\log \frac{2}{3} = \log 3 - \log 2$$
$$= 0{\cdot}176091$$

3. *Definition of the term "absorption relation."*—It has already been stated (see previous page) that the more concentrated a coloured liquid, the greater its absorbing power, the smaller, therefore, is the width of the stratum required to reduce the intensity of the light passed through it to one-tenth of its initial value. As the extinction-coefficient is, by definition, the reciprocal of the thickness of the stratum required to bring about this result, it follows that the greater the concentration of the solution, the greater will be the extinction-coefficient; in other words, the extinction-coefficient ε and the concentration c are proportional. Let c and c' represent the concentration of two coloured solutions, of which the extinction-coefficients are ε and ε' respectively, then

$$c : \varepsilon = c' : \varepsilon'$$

and

$$\frac{c}{\varepsilon} = \frac{c'}{\varepsilon'} = A$$

i.e. the relation of the concentration of a coloured solution to its extinction-coefficient is a constant, represented by A, and termed the "Absorption-relation" (*Absorptionsverhältniss*, Vierordt). Upon the determination of this constant rests Vierordt's method of quantitative spectrophotometric analysis. If we have, in the case of a solution of a particular body, determined by analysis its concentration c, and then with the spectrophotometer determined its extinction-coefficient *for a particular spectral region*, and thus obtained the value of A, we can find out how much of the same substance is contained in a solution of unknown strength (c') by merely determining ε', according to the equation:

$$c' = A\varepsilon'$$

It is usual to determine the value of the constant A of any coloured body under examination for, at least, two spectral regions. The reasons for this practice will appear in the sequel.

(b) The actual methods of spectrophotometry.—The elementary theoretical discussion of the theory of spectrophotometry which has preceded has shown that, as developed by Vierordt, it resolves itself into the determination of the extinction-coefficient and of the absorption relation of coloured bodies, and that the optical investigation is concerned with, and confined to, the determination of the value of ϵ. We have now to consider the two principal methods by which this determination can be effected.

Vierordt's method.—For the determination of the extinction-coefficient according to the original method of Vierordt, any good spectroscope of the type introduced by Bunsen for laboratory purposes may be employed, provided certain modifications and additions are made. The most essential of these modifications consists in replacing the usual single slit of the collimator by a double slit, *i.e.* by a slit composed of two independent halves—an upper one and a lower one—each of which is controlled by a micrometer screw provided with a divided circle or drum, so that the width of each half of the slit may be ascertained by direct reading (see Fig. 26). In so-called symmetrical slits,

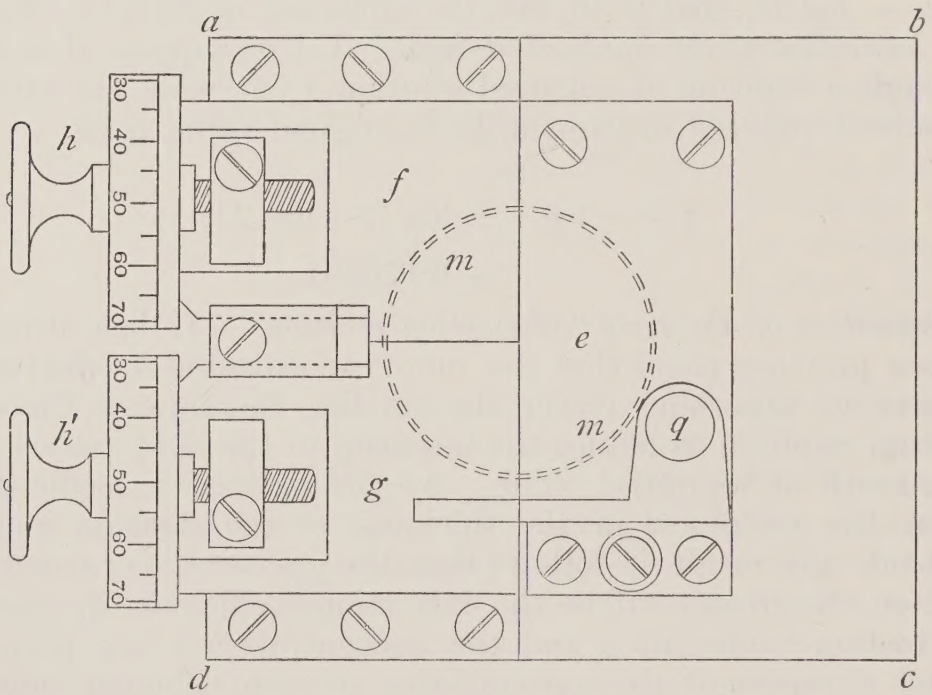

FIG. 26.—Double slit employed in Vierordt's method of spectrophotometry, as adjusted to their spectrophotometers by the Brothers Krüss of Hamburg.

both edges of the slit move symmetrically. When the two halves of such a slit are of the same width, *if the illumination be uniform*, the observer, on looking through the telescope of the spectroscope, observes two superposed spectra of equal brightness. If one slit be narrower than the other, the illumination of the corresponding spectrum will be diminished in proportion.

The second modification which has to be made in the ordinary spectroscope consists in substituting for the usual eyepiece, one which is provided with a slit for isolating any desired region of the spectrum, the remainder of the spectrum being concealed from view. In Vierordt's original instrument this slit was formed by two lateral shutters, moving in the focal plane of the eyepiece, which could be approximated to any desired extent. This simple contrivance has been perfected by Hüfner, and adapted to his beautiful spectrophotometer. A very ingeniously contrived and readily adjusted slit has been devised by the Brothers Krüss of Hamburg, and adapted to the spectrophotometers made by

their firm. Whatever the precise form of the slit in the eyepiece, it must permit of the isolation of a perfectly defined spectral region, and of the precise determination of the limits of that region, these being expressed in wave lengths.[1] For all coloured solutions there are regions in which the absorption of light is peculiarly distinctive, and which are specially favourable to the determination of the coefficient of extinction. In the case of oxyhæmoglobin, Hüfner has in his most recent researches selected a part of the region between the two absorption-bands (λ 550–λ 540) and a part of the region lying within the second band (λ 542·5–λ 531·5).

There remains to be described an absolutely essential accessory to the spectroscope, without which it would be impossible to determine the spectrophotometric constants. This is a specially contrived glass trough, for holding the solutions to be investigated, the anterior and posterior walls of which are formed by two perfectly parallel glass plates. Two forms of this trough are shown in Fig. 27, whilst Fig. 28 exhibits a trough mounted on its

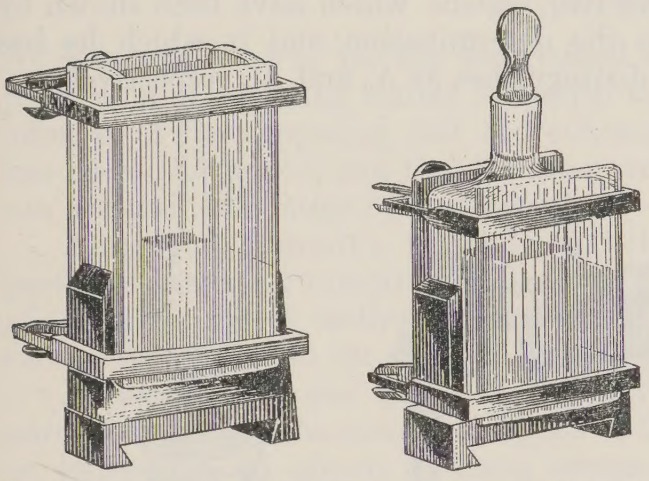

FIG. 27.—Glass troughs for containing the liquids to be examined by the methods of spectrophotometry.—After Krüss.

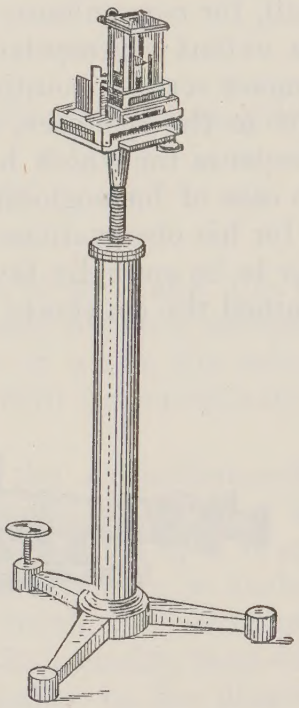

FIG. 28.—Trough mounted on stand, as used in spectrophotometry.—After Krüss.

stand, the stand permitting of the trough being easily and gradually lowered or raised, and of its being accurately levelled. The inner surfaces of the parallel glass plates of the little trough are exactly 11 mm. apart. A glass cube (called after the person who suggested its use, *der Schulz'sche Glaskörper*) exactly 10 mm. broad, and half the height of the interior of the trough, rests on the floor of the latter, so that the anterior and posterior surface of the cube shall be parallel with the glass plates of the trough (Fig. 29). When the coefficient of extinction of a coloured liquid is to be determined, such a trough is filled with it. When light passes through the lower half of the trough, it must traverse a stratum of coloured liquid 1 mm. in thickness, whilst light passing through the upper half traverses a stratum 11 mm. thick. In the latter case, the light is subjected to the absorbing action of a layer of

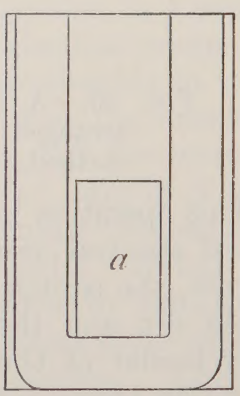

FIG. 29.—Section of glass trough with the *Schulz-'sche Glaskörper, a, in situ* (schematic).—After Krüss.

[1] The reader who wishes to understand the details which are necessary for practical work in spectrophotometry is advised to read in the first instance a useful, indeed almost

coloured liquid 1 cm. broader than that which is contained in the lower half of the trough, and this is for spectrophotometric purposes exactly equivalent to interposing a stratum 1 cm. broad in the path of the light impinging on one (the upper) half of the slit, and no coloured liquid in the path of the light reaching the other half.

Spectrophotometric measurements are invariably made by the aid of artificial light. Hitherto, oil or petroleum lamps have been used for this purpose, but lately Hüfner has adopted a gas lamp fitted with an Auer incandescent burner.

We are now in a position to complete our explanation of Vierordt's method. We shall assume that a spectrophotometer, such as has been described, is at the disposal of the observer. The lamp is lighted and the height of the flame adjusted, so as to equally illuminate the two halves of the double slit; this is seen to be the case when with equal widths of the slits two superposed spectra of exactly equal brightness are seen. The two halves of the slit are then opened to the extent which is thought advisable; we shall, for convenience of description, suppose that they have been opened to the extent represented by the index on the two divided circles of the micrometer screws, pointing to the division 100. The observer then arranges the slit in the eyepiece, so as to isolate and measure precisely the region of the spectrum for which he desires to determine the coefficient of extinction. In the case of hæmoglobin, of oxyhæmoglobin, and of CO-hæmoglobin, he will select for his observations one of the two regions which have been shown by Hüfner to be specially favourable to the determination, and in which he has determined the constants which he distinguishes as A_0 and A'_0 respectively.

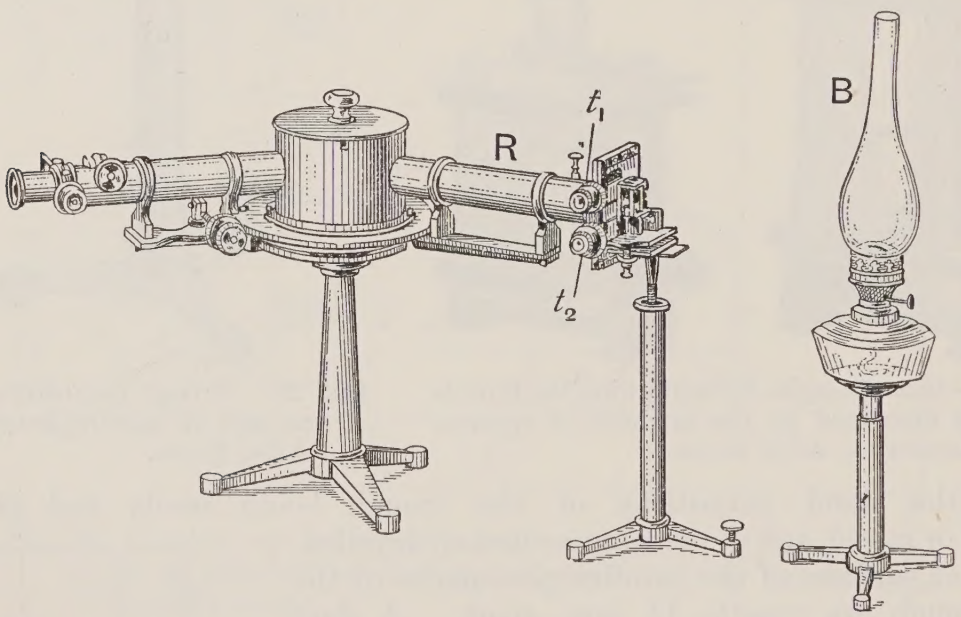

FIG. 30.—A spectrophotometer with absorption trough and lamp as arranged for spectrophotometric determinations by Vierordt's method.

This operation having been effected, he will again observe whether the two limited spectral areas appear to be of precisely equal brightness. If this is the case, the trough containing the coloured liquid is brought in front of the double slit, and the height of the former is carefully adjusted, so that the upper border of the glass cube appears as a line exactly coinciding with the separation between the upper and the lower spectral strips.

indispensable, book by Dr. Gerhard Krüss and Dr. Hugo Krüss, entitled "Kolorimetric und quantitative Spektralanalyse, etc.," Hamburg u. Leipzig, 1891. Though specially written for those who intend to work with Hüfner's instrument, an accurate though very succinct account of spectrophotometry is contained in a pamphlet entitled "Anleitung zum Gebrauche des Hüfner'schen Spectrophotometers, etc.," von Eugen Albrecht, Universitäts-Mechaniker in Tübingen: Tübingen, 1892. Subsequently, all Hüfner's papers on spectrophotometry should be studied.

We shall suppose this result to have been attained, and next direct our attention to the relative illumination of the two spectral areas under examination. It will be at once seen that the interposition of the absorption-cell has brought about a great difference in this respect. The upper spectrum is seen to be much less bright than the lower, the difference depending upon the amount of colouring matter in solution. Unless the concentration be excessive, we can restore the equality of illumination of the two superposed spectral areas by narrowing the lower slit. This is done with great care until we are convinced that the luminous intensity is the same in both, or that we have secured the greatest attainable equality (see, below, the discussion of the objections to Vierordt's method). We have then merely to read the division on the divided circle of the micrometer screw of the lower half of the slit. Supposing we find that the width of the lower slit is represented by division 20, whilst the width of the upper slit remains at 100, then the former number represents the percentage of unabsorbed light. We have seen that the extinction-coefficient ϵ can be determined by the formula—

$$\epsilon = -\log I', \text{ where } I'$$

represents the unabsorbed light. By a table of logarithms, or more quickly by special tables, we find that in our case—

$$\epsilon = -\log \tfrac{20}{100} = \log 100 - \log 20$$
$$\epsilon = 0{\cdot}69897$$

As has been shown, having determined ϵ, we may, if we know the precise proportion of colouring matter contained in the coloured solution, calculate the value of A; or supposing that the substance is one of which the value of A has been determined, and that we are unacquainted with its concentration, we can ascertain the latter by the formula $c = A\epsilon$.

Although Vierordt's method of determining the extinction-coefficient possesses historical interest, and its study is the natural introduction to that of the more perfect methods which have been suggested by it, it is open to serious objections, to the principal of which reference may here be made.

However wide one slit may be, and however much the other may be narrowed, it is, in the case of solutions of high colorific intensity, most difficult, or impossible, to obtain by these means alone equality in the illumination of the spectra; and accordingly Vierordt frequently had recourse to the use of smoke-tinted glass plates (*Rauchgläser*) of previously determined absorptive power, these being interposed in the path of the light which had not traversed the coloured solution. There are unquestionably theoretical and practical objections to this mode of proceeding. The principal objection to Vierordt's method is, however, a fundamental one, namely, that no absolute comparison is possible between spectra obtained with slits varying considerably in width. The more the slit of a spectroscope is widened, not only does the amount of light admitted increase and the spectrum become brighter, but the more and more impure does it become, *i.e.* the greater the admixture of light of different wave lengths in any region of the spectrum. But the accurate determination of the coefficient ϵ is only possible with monochromatic light. It has been sought to diminish the error due to the cause just referred to by substituting for the original double slit of Vierordt one of which both edges move symmetrically, so that the centre of the slit remains in a constant position. Although, doubtless, the error is reduced in this way, it is not entirely corrected.

Although Vierordt's method of determining the value of the co-efficient ϵ will probably fall in future into disuse, his great merit of having been the first to work out a method of spectrophotometry admitting of considerable accuracy, and of having discovered and established its applicability to the quantitative analysis of colouring matters, will always endure.

Hüfner's method.—This method, which has been made more and more

efficient by the long-continued labours of its author, differs from Vierordt's in the mode by which the equalisation of the intensity of two beams of light is brought about, the difference in mode requiring a spectrophotometer which differs in important respects from the instrument already described.

In Hüfner's spectrophotometer there is a single slit, the width of which, after it has been once adjusted, is never varied.

The light which reaches one-half of this slit has been polarised by a small Nichol's prism (the polariser), whilst that which reaches the other half (which in the determination of the value of ϵ passes through the thicker stratum of coloured liquid) is unpolarised. When these two beams of light fall upon the refracting prism of the spectroscope, they are refracted and furnish two superposed spectra, of which that corresponding to the polarised beam is naturally much less intense than the other. Before making any observations of ϵ, the two spectra must be equalised, this being done by interposing a wedge of smoke-tinted glass in the path of the unpolarised beam. Equality of both spectra having been obtained, if a coloured medium be placed in the path of the unpolarised beam, its spectrum will be correspondingly reduced. Equality is, however, restored by rotating a second Nichol's prism (the analyser) which is in the path of the beams issuing from the refracting prism, and the rotation of which diminishes the intensity of the polarised beam alone. When equality in the illumination of both spectra has been restored, the angle (ϕ), through which the analysing Nichol has been rotated, is measured in two opposed quadrants of a divided circle provided with a vernier, and from the value of ϕ that of I' is calculated.

If the original intensity of the light = 1, and the intensity of the unabsorbed light which has traversed the coloured medium be represented by I', then

$$I' = \cos^2\phi\,;$$

If the layer of coloured liquid investigated be always = 1 (*e.g.* 1 cm.), then

$$\text{as } \epsilon = -\log I',$$
$$\epsilon = -\log \cos^2\phi$$

The following example will illustrate the mode of procedure and the steps of the calculation in an actual experiment for the determination of the extinction-coefficient of blood, carried out with Hüfner's spectrophotometer:—

1 c.c. of defibrinated blood of the ox was diluted to 160 c.c. with a 0·1 per cent. aqueous solution of Na(OH). The absorption-trough was filled with some of the perfectly clear red liquid thus obtained. The spectral region (r), for which ϵ was determined, was one of the two in which Hüfner has, in his most recent experiments, determined the constant A of oxyhæmoglobin (*i.e.* a portion of the region between the bands α and β of oxyhæmoglobin).

$$r = \lambda 557{\cdot}5 - \lambda 568{\cdot}7$$

(Mean of ten measurements) $\phi = 61^\circ{\cdot}87$

Converting the decimal fractions of a degree into seconds $\phi = 61^\circ 52'$

It has been stated that with Hüfner's spectrophotometer

$$I' = \cos^2\phi$$
$$\text{and } \epsilon = -\log \cos^2\phi$$

In the above experiment

$$\epsilon = -\log \cos^2 61^\circ 52'$$
$$'' = -2 \log \cos 61^\circ 52'$$
$$'' = -2\,(0{\cdot}67350 - 1)$$
$$'' = -1{\cdot}34700 + 2$$
$$'' = 0{\cdot}653$$

Hüfner's spectrophotometer is an instrument of so much importance to the physiologist who intends to work at spectrophotometry, that a short description of the arrangements of its several parts appears desirable.

The instrument as a whole, as well as the stand carrying the absorption-trough and the lamp, are shown in Fig. 31.

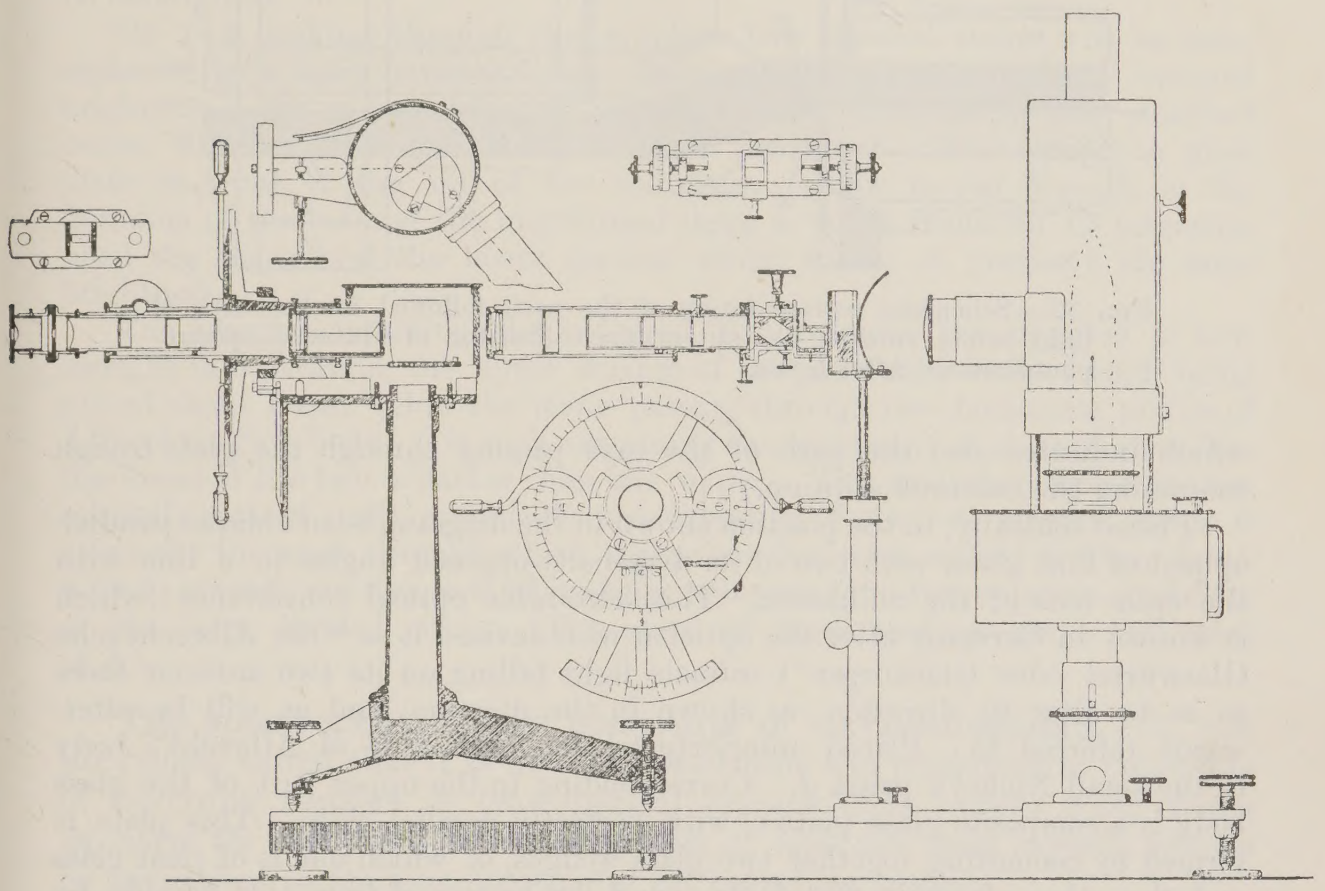

FIG. 31.—Hüfner's spectrophotometer, as made by Albrecht.

The spectrophotometer, the stand for the trough, and the lamp, rest upon the optical bench which forms the base for the whole. The position of the spectrophotometer is constant; the trough-stand and the lamp move along a slide, and can be placed at any required distance. During the actual experiment, the anterior edge of the trough is in close contact with the anterior part of the collimator. The lamp (which in the models recently and at present constructed is a gas lamp provided with an Auer incandescent burner) is for actual work placed at a distance of 24 to 25 cms. from the distal end of the collimator. The lamp is fitted with a positive lens the focus of which is made to correspond with the brightest part of the flame, so that perfectly parallel rays fall upon the absorption-trough. The latter is in all respects similar to the one used in Vierordt's method.

Turning our attention to the spectrophotometer, see Fig. 31, it is seen to be composed of a three-footed stand, furnished with levelling screws, the stand supporting the platform on which is fixed the dispersing prism, which is enclosed in a metallic case. To the right is seen the collimator and to the left the telescope.

1. *The collimator.*—This is furnished with a *single* slit formed by the edges of two slides moving transversely, each of which possesses its own micrometer screw, furnished with an accurately divided drum. This arrangement enables a slit of a precisely known width to be obtained, and the slit can be widened or narrowed symmetrically,—so that its centre remains constant.

Unlike ordinary spectroscopes, Hüfner's spectrophotometer has, fixed to the

front of the slit, a metallic box enclosing the following optical parts. (In order to facilitate our description, these are shown in the following diagram, Fig. 32,

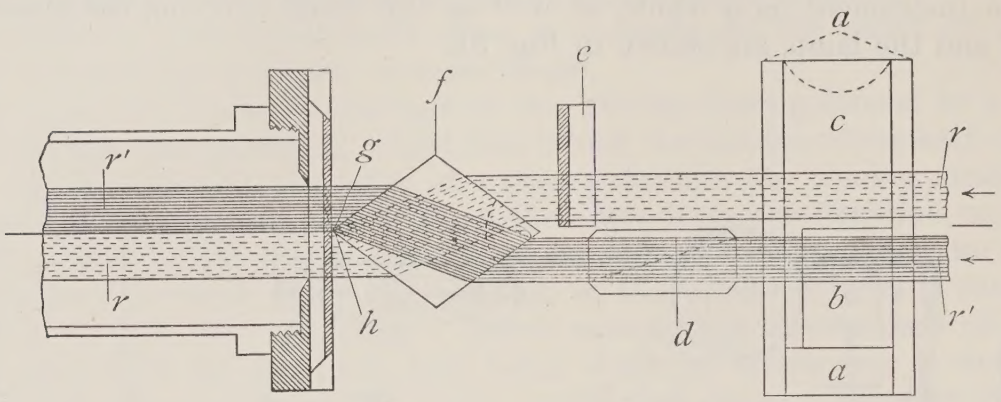

FIG. 32.—Schematic representation of the path followed by the rays of light before entering the slit of the collimator of Hüfner's spectrophotometer.—After Krüss.

which indicates also the path of the rays passing through the glass trough containing the coloured solution.)

Placed centrally, in the position shown in the diagram, is an oblique parallelopiped of flint glass, with two of its diagonally-opposed angles in a line with the optic axis of the collimator. This admirable optical contrivance (which is known in Germany after the optician who devised it as "der Albrecht'sche Glaswürfel oder Glaskörper") refracts light falling on its two anterior faces so as to alter its direction, as shown in the diagram, and as will be afterwards referred to. Placed anteriorly to the lower half of Albrecht's body is the small Nichol's prism *d*. Corresponding to the upper part of the glass body is a composite glass plate *e*, with perfectly parallel sides. This plate is formed by cementing together two glass wedges, of which one is of clear glass and the other of smoke-tinted glass, and can be moved from side to side by means of a special arrangement. According to the position of this plate it will absorb more or less light. The purposes of these various parts are sufficiently obvious from the diagram; *aa* represents the absorption-trough for containing the coloured liquid to be spectrophotometrically investigated. In the lower half of the trough is seen the Schulz's cube (*b*); *r* and *r'* represent two parallel beams of light falling on the anterior surface of the trough. The lower beam (*r*) traverses in its path the Nichol prism (*d*), and is polarised; falling then on the adjacent surface of the parallelopiped, it is deviated so as to fall upon the upper half of the slit. The upper beam may or may not meet in its path the composite plate *c* previously referred to, and to which reference will again be made. This beam is so deviated as to fall upon the lower half of the slit. After traversing the structures just described, two beams of light fall upon the slit—a polarised beam on the upper half and a non-polarised beam on the lower.

2. *The telescope.*—A very ingenious arrangement, which is indicated by a separate drawing in the centre of Fig. 31, permits of the precise position of the telescope in reference to the prism being determined, and consequently of the most accurate determination of the position of any line in the spectrum. The reader is referred for details to Professor Hüfner's original description. At the distal end of the telescope is the object glass, next to it is a Nichol's prism, the rotation of which is measured on a graduated circle by the help of a vernier. In the focal plane of the eyepiece is a modification of Vierordt's eyepiece slit, permitting of any determined spectral region being exactly isolated. For further details as to the construction and adjustment of the spectrophotometer, the reader is referred to the original

sources of information. It is absolutely essential to work with Hüfner's spectrophotometer in a perfectly darkened room.

Before commencing photometric measurements, the observer will ascertain whether the analysing Nichol is in the position in which it allows the polarised beam to pass unabsorbed. He will then fill the absorption-trough, and isolate and measure the spectral region for which the extinction-coefficient is to be determined.

On now looking through the eyepiece two spectral strips will be seen, separated by a sharp horizontal line; these spectral strips will be of unequal brightness; the upper, being a portion of the spectrum of the polarised beam, will be much less luminous than the lower. The composite glass plate in front of the slit of the collimator is now moved inwards in the direction of the beam of the unpolarised light, so as to diminish its intensity, until the upper and the lower spectral strips appear of precisely the same brightness.

The trough containing the coloured liquid under investigation is now brought into position, the upper surface of the glass cube in the trough being placed about 1 mm. below the plane passing through two horizontal angles of Albrecht's glass body. On now examining the spectra, it is at once seen that the lower of the two is darker than the upper. The analysing Nichol is then carefully rotated until equality in the intensity of the two spectral strips is attained; the angle through which the prism has been moved is then determined; several, say five, sets of readings being made in two opposite quadrants of the large divided circle. The mean of these readings gives the value of ϕ.[1]

The spectrophotometric constants of oxyhæmoglobin.—It was previously stated that it is usual to determine the photometric constants of colouring matters in two spectral regions, *those regions being chosen in which the variations in the absorption of light are most rapidly affected by variations in the concentration of the colouring matter.*

The reasons for determining in the first instance at least two values for A (which we shall distinguish as A and A'), and subsequently, each time that a determination is made, ascertaining the value of ϵ in the same two regions (the two extinction-coefficients being distinguished as ϵ and ϵ', or in the case of oxyhæmoglobin as ϵ_0 and ϵ_0') are the following:—(1) If we know the value of A and A' for any body, we are able to make two independent estimations when determining the concentration of a solution of the same body of unknown strength, the one estimate acting as a check on the other. (2) The knowledge of the value of A and A', for each of two colouring matters co-existing in solution, is a necessary condition to being able to determine spectrophotometrically the amount of each constituent when occurring together. (3) In the case of oxyhæmoglobin, hæmoglobin, and CO-hæmoglobin, the quotient $\frac{\epsilon'}{\epsilon}$ is absolutely characteristic of each substance, and affords a valuable check on the purity of the colouring matter in solution and on the accuracy of the analysis.

Hüfner's most recent determinations[2] of the spectrophotometric constants of oxyhæmoglobin, made with his perfected spectrophotometer, have led to the results shown below. The two values of A are, as has

[1] Hüfner's spectrophotometer is constructed by, and can be obtained from, the original maker, Herr Eugen Albrecht, Universitäts-Mechaniker in Tübingen.

[2] Hüfner, "Photometrische Constanten des Oxyhämoglobins," *Arch. f. Physiol.*, Leipzig, 1894, Physiol. Abth., S. 134 *et seq.*

been previously stated, in the case of oxyhæmoglobin, distinguished by the symbols A_0 and A'_0; the first has been determined for the spectral region which lies between the two bands α and β of oxyhæmoglobin, and is limited by λ 554 and λ 565; the second has been determined for the spectral region which corresponds to the darkest part of the second (β) band of oxyhæmoglobin, and extends from λ 531·5–λ 542·5.

Spectrophotometric Constants of Oxyhæmoglobin[1] (*Hüfner*).

Limits of Spectral Region in which ϵ_0 and ϵ'_0 were determined.	Absorption relation A_0 and A'_0 corresponding to the two regions.
(R of ϵ_0) . . λ 554–λ 565	A_0 . . . . . 0·002070
(R of ϵ'_0) . λ 531·5–λ 542·5	A'_0 . . . . . 0·001312

From the above constants we are able, as has been shown (see p. 215), to determine the percentage of hæmoglobin in the blood with surprising accuracy. The further use of these constants will be referred to in explaining the mode of determining the relative amounts of hæmoglobin and oxyhæmoglobin coexisting in any sample of blood.

We have now to consider in some detail the light which spectrophotometry has shed on certain questions which possess great interest to the physiologist, and which have up to a certain point been already discussed in this article.

Hüfner and his pupil v. Noorden long ago noticed that the quotient $\frac{A'_0}{A_0}$, which is the same as $\frac{\varepsilon'_0}{\varepsilon_0}$, was remarkably constant, not only in the blood of animals of the same species, but in all, however widely separated in the animal scale.[2] Subsequent researches by Hüfner and his pupils, carried out with a much more perfect spectrophotometer than the one employed by v. Noorden and himself, more than confirm the earlier results in so far as the constancy of the quotient is concerned.

If the defibrinated blood of any animal, diluted with 150–160 parts of 0·1 solution of NaOH, or a solution in the same dilute NaOH of crystals of oxyhæmoglobin of approximately equivalent concentration, be thoroughly oxygenated by shaking with air and the values of ε_0 and ε'_0 be determined, it will be found that the quotient $\frac{\varepsilon'_0}{\varepsilon_0}$ will vary very slightly from 1·580. In very few determinations, out of a large number, was it as low as 1·578. So soon, however, as the blood commences to undergo any change, as, *e.g.*, a partial conversion into methæmoglobin, the coefficient is lowered.

[1] The values of A_0 and A'_0 given above differ materially from those which had been assigned to them previously by Hüfner and his pupil v. Noorden as a result of researches carried out with Hüfner's earlier and much less perfect spectrophotometer, and employing hæmoglobin which had been frequently recrystallised.

[2] v. Noorden's observations included the blood of man, the dog, the cat, the rat, the guinea-pig, and the owl. "Beiträge zur quantitativen Spektralanalyse, in besondere zu derjenigen des Blutes" (aus d. Lab. d. Prof. Hüfner in Tübingen), *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 9–35.

From the extraordinary constancy of this quotient some interesting conclusions may be legitimately drawn. (1) The constancy of the quotient in all animals affords presumptive evidence, amounting to absolute proof, that the iron-containing molecular group existing in hæmoglobin, upon which its colour, its light-absorbing power, and its capacity to combine with O, CO, and NO depend, is identical in all animals. The truth of this hypothesis is borne out by many weighty facts, *e.g.* the identity in chemical composition (as revealed by analysis) of the iron-containing products of the decomposition of hæmoglobin, whatever its source; the constancy in the proportion of O and CO which can combine with 1 grm. of hæmoglobin of different animals. (2) The constancy of the quotient (whether solution of crystallised hæmoglobin, or an alkaline solution made by diluting defibrinated blood with 0·1 per cent. vol. of Na(OH), or a liquid holding intact blood corpuscles in suspension, be investigated), shuts out the possibility of more than one colouring matter existing in the blood. It renders absolutely untenable the views of Bohr, who has assumed the existence of several hæmoglobins, possessed of different powers of combining with oxygen; and utterly disproves Hoppe-Seyler's hypothesis that the colouring matter of the corpuscles is distinct from hæmoglobin so as to deserve a special designation of arterin or phlebin, as the case may be.

(*b*) **The photographic spectrum.** — In the year 1878 the late Professor J. L. Soret, of Geneva, in his first memoir on the absorption of the ultra-violet rays of the spectrum by diverse organic substances,[1] announced the fact that diluted blood, when examined with the aid of a spectroscope provided with a fluorescent eyepiece, presented in the extreme violet, between Frauenhofer's lines G and H, an absorption-band which appeared to him to be slightly shifted towards the less refrangible end of the spectrum, when the blood solution was saturated with carbonic oxide. Soret subsequently[2] confirmed the accuracy of the above facts, employing the photographic method in his experiments, though he published none of his photographs. Since the date of the publication of Soret's short notes on this subject, d'Arsonval[3] has independently, and without referring to Soret's observations, described anew the extreme violet absorption-band of the blood-colouring matter, but without adding to the facts discovered by the Swiss observer.

The complete absence of all reference to Soret's scanty but interesting and suggestive observations, in text-books and treatises on physiology and physiological chemistry; and the fact, which my own observations soon elicited, that the absorption-band of Soret is even more distinctive of the blood-colouring matter than the absorption-bands in the visible spectrum which have hitherto engrossed the attention of observers, led me to study this absorption-band in more detail in hæmoglobin, its compounds and principal derivatives.[4] I

[1] J. L. Soret, "Recherches sur l'absorption des rayons ultra-violets par diverses substances," *Arch. d. sc. phys. et nat.*, Genève, 1878, pp. 322, 359.

[2] Soret, *ibid.*, 1883, pp. 194, 195, 204.

[3] A. d'Arsonval, *Arch. de physiol. norm. et path.*, Paris, 1890, Sér. 5, tome ii. pp. 340–346.

[4] A. Gamgee, "On the Absorption of the Extreme Violet and Ultra-Violet Rays of the Solar Spectrum by Hæmoglobin, its Compounds, and certain of its Derivatives," *Proc. Roy. Soc. London*, 1896, vol. lix. p. 276.

propose that the band in the extreme violet should henceforward be distinguished as the band γ, or the band of Soret, in the spectrum of oxyhæmoglobin.

Methods of demonstrating the band of Soret.—The limits of visibility of the solar spectrum correspond, as usually stated, with the H group of lines; here lies the arbitrary boundary which separates the extreme violet from the ultra-violet properly so called—that region which we can only see by interposing fluorescent media in the path of the rays (*e.g.* a fluorescent eyepiece), or by allowing the spectrum to fall on a fluorescent surface—the region which is best studied by the aid of photography.

Although Soret's band lies at the limit, but yet within the boundaries, of the visible spectrum, it is impossible to see it with the ordinary spectroscope, *i.e.*, unless this be provided with special devices. It has already been stated that it can be seen with any spectroscope, if we substitute a fluorescent for the ordinary eyepiece; a cell containing a dilute solution of æsculin must, however, be substituted for and placed in the position of the uranium glass plate of the eyepiece, uranium glass fluorescing most feebly in the light of the spectral region where the absorption-band under discussion is situated. It was, indeed, with the aid of his fluorescent eyepiece that Soret first discovered this band, though d'Arsonval asserts that it is impossible to see it in this way. Observations with the fluorescent eyepiece are, however, difficult and require experience. Still more difficult and unsatisfactory is the method, also suggested by Soret, and lately published as an original suggestion by d'Arsonval, of rendering this band visible by interposing a blue glass between the eye and the spectroscope. If the light be very intense the band is just perceptible to a person who is already acquainted with its position and characters through other methods of observation.

In order to demonstrate Soret's band and the absorption-bands in the ultra-violet of derivatives of the blood-colouring matter, I projected the spectrum of sunlight or of the positive pole of the electric arc on to a fluorescent screen, similar to those which have since come into common use in observations made with the X or Röntgen rays, *i.e.* a screen made by coating a white surface, such as cardboard, with barium platinocyanide.

In order to render absorption-bands of coloured liquids in the extreme violet and ultra-violet beautifully visible by this method, it is essential, however, to open the slit which intervenes between the source of light and the collimating lens *very widely*. In the highly luminous spectra thus obtained, though none of the spectral lines are visible, except perhaps H and K appearing blurred and indistinct, absorption-bands appear with remarkable distinctness and sharpness. The method is valuable, not only for purposes of demonstration, but for making preliminary observations prior to having recourse to photography. By its help I ascertained with correctness the position and characters of the extreme violet and ultra-violet absorption-bands of the acid compounds of hæmatin, of methæmoglobin, of hæmatoporphyrin, and of turacin. In no case where this method yielded negative results, was the presence of a band afterwards demonstrated by photography.

As few physiological laboratories possess a perfectly darkened optical

room provided with a heliostat for projecting a beam of sunlight into it, the following simple arrangement,[1] which requires merely an electric arc lamp and an ordinary laboratory spectroscope of the Bunsen type, may be adopted.[2] The telescope of the spectroscope is removed, and a beam from the + *pole* of the arc is allowed to fall on the slit of the collimator. The spectrum is focussed on a fluorescent screen, then the slit is opened very widely. If the spectrum be a continuous one (which is the case if it be that of the positive pole of the electric arc), the coloured solution is then interposed in the path of the beam falling on the slit.

The position and limits of Soret's band.—Defibrinated arterialised blood, diluted with from 400 to 600 volumes of distilled water, or still better with a similar amount of 0·1 per cent. solution of sodium hydrate,

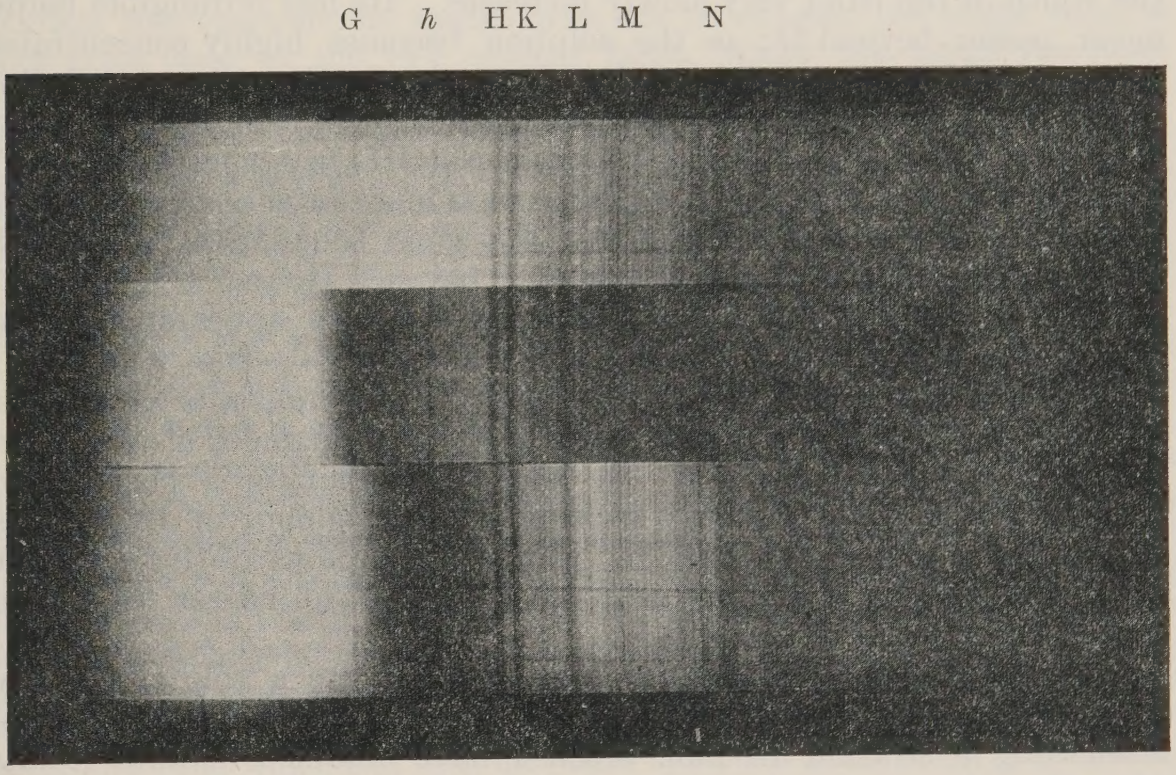

Fig. 33.—The photographic spectrum of hæmoglobin and oxyhæmoglobin.

furnishes solutions (containing *about* 1 part of oxyhæmoglobin in 3000 and 1 part in 5000 respectively) of a concentration suited for photographic investigations of the spectrum. With solutions of this strength (a stratum 1 cm. thick being placed in the path of the beam falling upon the slit of the collimator) Soret's band can be studied to perfection, though it can be well seen with solutions much more concentrated and much more dilute. The appearance and position of Soret's band in the spectrum of oxyhæmoglobin are shown in Fig. 33 along with that of reduced hæmoglobin.

Within fairly wide limits of concentration (the stratum examined being invariably 1 cm. wide), the limits and characters of Soret's band

[1] I employed this simple arrangement in demonstrating these bands in the violet and ultra-violet to members of the Internat. Physiological Congress, Bern Meeting, September 1895.

[2] Direct vision spectroscopes cannot be used, the absorption of the ultra-violet rays being very great in these instruments.

remain very constant, the increase in the amount of oxyhæmoglobin influencing more the intensity of the band than its width. In the case of solutions containing approximately the proportion of oxyhæmoglobin above mentioned (*i.e.* from 0·20 to 0·33 parts in 1000), the spectral region between F and G is absolutely unshaded. Soret's band is then seen, extending from λ 404–λ 434; *i.e.* it occupies the greater part of the spectral region intervening between G and H; the edges, however, uniformly shade away as far as these lines.

By examining a series of photographs of spectra obtained by interposing solutions of oxyhæmoglobin of very different concentrations, I have determined that the mean ray absorbed does not, as Soret thought, coincide with *h* (λ 410·1), but is decidedly on the red side of that line, corresponding to λ 414.

When the concentration of the solution of oxyhæmoglobin increases, the width of the band very slowly increases. Its less refrangible border never passes beyond G; as the solution becomes highly concentrated, the band widens perceptibly, and *it does so in the direction of the ultra-violet.* With a solution made by diluting 1 volume of blood to the volume of 250 (water or 0·1 per cent. solution of Na(OH) being employed as the diluent), the absorption-band, though much more intense than with the more dilute solutions, retains almost the same boundaries, its shadowy borders approaching, but not passing beyond, G and H. With a solution containing 1 part of blood in 100, the appearances differ remarkably from those previously referred to. The solution is transparent for light from F to nearly G; it transmits light with difficulty from L to N (λ 381·9–λ 350·01); the remainder of the ultra-violet is completely absorbed. A solution containing 5 per cent. of defibrinated blood (or about 6·5 parts of oxyhæmoglobin in 1000 parts) absorbs the whole of the violet and ultra-violet regions of the spectrum, with the exception of a region between F and G, but nearer the former (λ 460–λ 490).

It remains to be considered with how dilute a solution of oxy-hæmoglobin a photographic record of Soret's band can be obtained. Examining a stratum 10 mm. broad I have obtained definite results, when the solution contained somewhat, but not much, less than 1 part of oxyhæmoglobin in 10,000.

No colouring matter yet investigated exhibits the intense absorption-band between G and H which is characteristic of hæmoglobin and its compounds. Several substances (carmine, picro-carmine, and the colouring matter of alkanet root) exhibit absorption-bands in the visible part of the spectrum which bear a superficial resemblance to those of oxy-hæmoglobin. The spectrum of none of these colouring matters exhibits, however, any absorption in the extreme violet or the adjacent ultra-violet.

The researches which I have conducted have shown that the band of Soret depends on the iron-containing group existing in the hæmoglobin molecule, yet not upon its iron. The variations in character and position which this band exhibits in the various compounds and derivatives of hæmoglobin will be referred to under each.

Hæmoglobin (Reduced Hæmoglobin).

Synonym, "Purple Cruorin."

Historical note.—Fully two years had passed since the date of Hoppe's publication (1862) of his observations on the spectrum of blood, before it was shown that the oxygen which enters into combination with hæmoglobin has a fundamental influence on its spectrum. It was on the 16th of June 1864 that Professor Stokes [1] communicated to the Royal Society the interesting observation that when diluted blood is treated with certain reducing agents, the colour of the liquid and its spectrum undergo remarkable changes; the former loses its bright red appearance, becoming darker in tint, whilst the absorption-bands α and β are replaced by a single band which we may designate the band γ, which appears less deeply shaded and with less defined edges, and which extends from D nearly to E. If, now, the solution which exhibits this spectrum be shaken with air or oxygen, the single band at once gives place to the two original bands, whilst the liquid reacquires more or less of its primitive florid-red colour. The process of reduction and oxidation may be repeated many times in succession.

From his experiments, Stokes concluded that "*the colouring matter of blood, like indigo, is capable of existing in two states of oxidation, distinguishable by a difference of colour and a fundamental difference in the action on the spectrum. It may be made to pass from the more to the less oxidised state by the action of suitable reducing agents, and recovers its oxygen by absorption from the air.*" [2]

The researches of Magnus, Lothar Meyer, and Claude Bernard had shown that the blood holds in solution an amount of oxygen greatly in excess of that which could exist in a state of simple solution, but that this oxygen exists in a condition which permits of its being extracted from the blood by boiling in a Toricellian vacuum, as well as by the action of carbonic oxide. Hoppe-Seyler, having succeeded in crystallising oxyhæmoglobin, and, by means of its optical properties, having identified it with the colouring matter as it exists in the living blood, was able to show that a solution of crystallised oxyhæmoglobin behaves towards reducing solutions in the same manner as diluted blood; that, like blood, it yields oxygen when boiled *in vacuo*, and that the blood-colouring matter thus deprived *in vacuo* of its loosely combined or respiratory oxygen manifests the absorption-band which had been described by Stokes as the result of reduction.

The further steps in the growth of our knowledge of reduced hæmoglobin will be more conveniently referred to in discussing the chief facts with which we are acquainted relative to this remarkable body.

Methods of effecting the reduction of oxyhæmoglobin to reduced hæmoglobin.—In nearly all experiments on the reduction of oxyhæmoglobin, diluted blood may be substituted for a solution of the pure blood-colouring matter, it having been shown by the spectrophotometric and chemical researches of Hüfner that, both in respect of their power of absorbing light and of the influence of reducing agencies upon them, the two solutions possess identical properties. Instead, however, of employing pure distilled water as a diluent, it is advisable to use, according to Hüfner's plan, a 0·1 per cent. solution of sodium hydrate. A diluted solution of blood prepared in this way is free from all turbidity, and therefore more transparent than a pure aqueous solution, and undergoes putrefactive alterations more slowly.

[1] "On the Reduction and Oxidation of the Colouring Matter of the Blood," *Proc. Roy. Soc. London*, 1864, vol. xiii. pp. 353–364; *London, Edinburgh, and Dublin Phil. Mag.*, London, 1864, vol. xxviii. pp. 391–400.

[2] Stokes, *op. cit.*, p. 357, par. 8.

1. **By the action of reagents exerting a reducing action.**—It is an essential condition which all reagents to be employed in the reduction of oxyhæmoglobin to hæmoglobin must fulfil, that they do not act destructively on these substances, as is the case with acids and salts possessed of an acid reaction.

Ordinary solutions of ferrous sulphate or stannous chloride cannot, for instance, be employed, as they instantly lead to a decomposition of the blood-colouring matter. The first and still the most generally employed reducing agents, the use of which dates back to the researches of Stokes on the blood-colouring matter, are ferrous and stannous salts and the alkaline sulphides. Utilising the well-known property of citric and tartaric acids to prevent the precipitation of the salts of iron and tin by ammonia and the alkaline hydrates, Stokes indicated easy methods of preparing active solutions of ferrous and stannous salts for the study of the reduction of oxyhæmoglobin.

(*a*) *Alkaline solutions of ferrous salts* (*Stokes' reagent*).—To a solution of a ferrous salt (usually ferrous sulphate or ferrous ammonium sulphate) ($Fe(NH_4)_2(SO_4)_2.6H_2O_1$), citric or tartaric acids or one of their alkaline salts is added, and then ammonia, until the reaction is alkaline. A light green solution is thus obtained, which rapidly darkens in the presence of air by the absorption of atmospheric oxygen. Such a solution, which must be freshly prepared, exerts a powerful and exceedingly rapid reducing action on oxyhæmoglobin, *even in the cold.* Alkaline ferrous solutions possess the disadvantage, in proportion as they absorb oxygen and become oxidised, of becoming coloured, and absorbing the more refrangible rays of the spectrum, interfering, therefore, with the accurate study of the specific absorption due to the colouring matter.

(*b*) *Alkaline solutions of stannous salts.*—These are made as described under *a*, by substituting a stannous (usually $SnCl_2$) for a ferrous salt. As they do not become coloured on salt being oxidised, these solutions do not interfere with the accurate study of the absorption of the violet rays. Like the analogous ferrous solutions, those containing tin rapidly reduce hæmoglobin *even in the cold.*

(*c*) *Solutions of the alkaline sulphides.*—Solutions of these salts (ammonium sulphide being almost invariably employed) effect the reduction of oxyhæmoglobin, but much more slowly than is the case with *a* and *b*, and their action is greatly accelerated by heat. Solutions of ammonium sulphide for this purpose should be freshly prepared, and be protected from the action of atmospheric oxygen and light, which bring about chemical changes, and cause them to assume a yellow colour and to absorb the violet end of the solar spectrum.

Solutions of the crystalline sodium monosulphide (Na_2S) cannot be employed with advantage as reducing agents for oxyhæmoglobin, as, according to my experiments, they lead at once to the formation of sulphomethæmoglobin, so that the pure spectrum of reduced hæmoglobin cannot be observed.

(*f*) *Agitation with finely-divided iron,*[1] or with metallic iron reduced by hydrogen—the so-called officinal *ferrum redactum.*[2]

[1] Rollett, "Versuche ueber thatsächliche und vermeintliche Beziehungen d. Blutsauerstoffes," *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1866, Bd. lii. Abth. 2, S. 246 *et seq.*

[2] Ludwig und Schmidt, "Das Verhalten der Gase welche mit dem Blut durch den reizbaren Säugethiermuskel strömen," *Sitzungsb. d. k. Sächs. Gesellsch.*, Leipzig, 1868, Bd. xx. S. 12–72.

(*g*) *Solution of sodium hydrosulphite* ($NaHSO_3$).—By the action of metallic zinc on a solution of sodium sulphate, in the absence of oxygen, a solution of intense reducing power is retained. Such a solution, which instantly decolorises indigo and litmus, reduces oxyhæmoglobin.[1]

Methods of determining the percentage of hæmoglobin have been based on this reaction,[2] [3] though they have been abandoned as unreliable.[4]

(*h*) *Solution of hydrazin and its salts.*—It was pointed out by Curtius[5] that a solution of a salt of hydrazin reduces solutions of oxyhæmoglobin with great rapidity; and Hüfner[6] afterwards employed an aqueous solution of hydrazin hydrate to effect the reduction of concentrated solutions of oxyhæmoglobin, the advantages of this reducing agent being that the only products of its decomposition are nitrogen and water, as shown in the following equation:—

$$\underset{\text{(hydrazin hydrate)}}{H_2N—NH_2.H_2O} + O_2 = N_2 + 3H_2O.$$

2. **By taking advantage of the reducing action exerted by products of putrefaction.**—The solutions of oxyhæmoglobin, or of diluted blood, is set aside in sealed tubes, when, especially at temperatures approaching 40° C., reduction rapidly occurs. It is worthy of remark that, whilst oxyhæmoglobin or its solutions very rapidly undergo change at temperatures above 0° C., such is not the case with reduced hæmoglobin, which may be kept for many years in sealed tubes in the presence of putrefactive bacteria and the products of their activity. On opening the tubes and agitating with air, oxyhæmoglobin is at once formed, and under favourable conditions may be crystallised.

3. **By taking advantage of the conditions which favour the "dissociation" of the compound of O_2 with hæmoglobin.**—(*a*) By boiling in a "Toricellian" or barometric vacuum; (*b*) by subjecting diluted blood or a solution of oxyhæmoglobin to the action of a long-continued stream of a neutral gas, such as hydrogen, nitrogen, or nitrous acid.

4. **By temporarily arresting the circulation through a sufficiently transparent part of the animal body.**—It was first pointed out by Vierordt,[7] that the spectrum of oxyhæmoglobin can be satisfactorily demonstrated by bringing two fingers (preferably the fourth and fifth) close together and passing a beam of sunlight through the comparatively thin layer of tissues at the boundaries of the adjacent fingers. He further pointed out that, on placing caoutchouc rings at the base of the first phalanges, after an interval varying between 40 and 300 seconds (?), the two bands of oxyhæmoglobin became replaced by the single band indicative of reduced hæmoglobin.[8]

[1] Schützenberger and Risler, "Recherches sur le pouvoir oxydant du sang," *Compt. rend. Acad. d. sc.*, Paris, 1873, tome lxxvi. pp. 440–442, and pp. 1214–1216.

[2] Rollett, *loc. cit.*

[3] Ludwig and Schmidt, *loc. cit.*

[4] Quinquaud, "Sur un procédé de dosage de l'hémoglobine dans le sang," *Compt. rend. Acad. d. sc.*, Paris, 1877, tome lxxvi. p. 1489.

[5] *Journ. f. prakt. Chem.*, Leipzig, 1889, Bd. xxxix. S. 27.

[6] "Bestimmung d. Sauerstoffscapacität d. Blutfarbstoffs," S. 156.

[7] "Das Hämoglobinspectrum am lebenden Menschen," *Ztschr. f. Biol.*, München, 1876, Bd. xi. S. 188; and "Die Sauerstoffzehrung der lebenden Gewebe," *ibid.*, 1878, Bd. xiv. S. 422.

[8] Refer to the following papers by A. Hénocque, "Étude spectroscopique du sang à la surface sous-unguéale du pouce," *Compt. rend. Soc. de biol.*, Paris, Sér. 8, tome i. p. 671; and also "Notes complementaires," *ibid.*, p. 700. According to this author, the average time of reduction, when the circulation through the thumb is arrested, varies between fifty-five and sixty-five seconds.

Preparation of crystallised hæmoglobin (reduced hæmoglobin).—It was shown almost simultaneously and independently by Kühne[1] and by Rollett[2] that highly concentrated solutions of pure oxyhæmoglobin may, after reduction, be made to crystallise, and that the crystals of reduced hæmoglobin, though differing in colour and spectroscopic characters from the oxygen compound, are essentially identical with it in crystalline form. Kühne explained that the difficulty which is encountered, when attempting to crystallise reduced hæmoglobin, depends upon its very great solubility.

Hoppe-Seyler was unable to crystallise reduced hæmoglobin;[3] and Hüfner[4] in 1880 published a note, in which he announced that he had succeeded in obtaining crystals of reduced hæmoglobin, though he neither then nor afterwards referred to the much more complete account published by Kühne fifteen years earlier.

In order to obtain crystals of reduced hæmoglobin for microscopic examination, a pure and highly concentrated solution of oxyhæmoglobin in *very* dilute ammonia is placed in a gas chamber, and a stream of chemically pure and thoroughly dried hydrogen is passed over it; as the solution evaporates crystals separate.[5]

Nencki and Sieber have obtained large quantities of crystals of reduced hæmoglobin by reducing concentrated solutions of pure oxyhæmoglobin of the horse through the agency of putrefactive bacteria, then adding a sufficient quantity of 25 per cent. alcohol and exposing to cold. The method which I employed more than twenty years ago, and which appears to me to offer some advantages, is to place a magna of pure oxyhæmoglobin crystals with a small quantity of the mother liquor from which they have separated in a glass tube, so as nearly to fill the latter, and then to seal it. The tube is heated for some days in an incubator at about 35° C., and is then set aside in a cool place. After some weeks of exposure to a winter temperature, the tube is found to contain large quantities of crystallised and perfectly reduced hæmoglobin.

No one has hitherto attempted to recrystallise reduced hæmoglobin, though, with the conveniences at present at the disposal of the scientific chemist, the process would present little difficulty.

Characters of the crystals of reduced hæmoglobin.—In form they are, as has been said, essentially identical with those of the oxygen compound, and like these are doubly refracting. Hüfner often obtained crystals 1 mm. long; and Nencki and Sieber, working with horses' blood, obtained crystals, mostly in the form of hexagonal plates, 2 or 3 mm. in diameter. They are pleochromatic, appearing of a dark red colour in some lights, and exhibiting a bluish or purple tinge in others.

[1] "Das Vorkommen und die Ausscheidung des Hämoglobins aus dem Blute," *Virchow's Archiv*, 1865, Bd. xxxiv. S. 423–436.

[2] *Loc. cit.*

[3] *Med. Chem. Untersuch.*, Berlin, S. 373.

[4] "Ueber krystallische Hämoglobin," *Ztschr. f. physiol. Chem.*, Strassburg, 1880, S. 383. It is singular that Nencki and Sieber, in an interesting and really valuable paper, should in 1887 have published *again*, as a new discovery, the obtaining of crystals of reduced hæmoglobin, though they subsequently disclaimed all priority (see M. Nencki and N. Sieber, "Venöse Hämoglobinkrystalle," *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1886, Bd. xix. S. 128 and 410).

[5] Kühne, *op. cit.*

When the blood crystals of horses' blood are prepared in closed vessels, it happens very frequently that large quantities of hexagonal tables of a *dark red colour* are found mixed with the well-known ordinary prisms. If a drop of the liquid in which the crystals are suspended be examined with the microscope, *without a cover-glass*, the hexagonal plates are observed rapidly to liquefy, and simultaneously bundles of fine, bright-red prismatic needles appear. Nencki long ago showed that the dark red hexagonal tables are crystals of reduced hæmoglobin, whilst the scarlet prisms are those of oxyhæmoglobin. Horses' blood appears peculiarly apt to give crystals of the reduced blood-colouring matter. In the preparation of the hæmoglobin of the horses' blood by ordinary methods, *i.e.* without special precautions in reference to the access of air, both forms of crystals are usually obtained.[1]

The Absorption of Light by Solutions of Reduced Hæmoglobin.

Colour of solutions: dichroism.—In thick layers, or in thin layers if concentrated, solutions of reduced hæmoglobin present a dark cherry-red colour, whilst very dilute solutions exhibit a green tint.

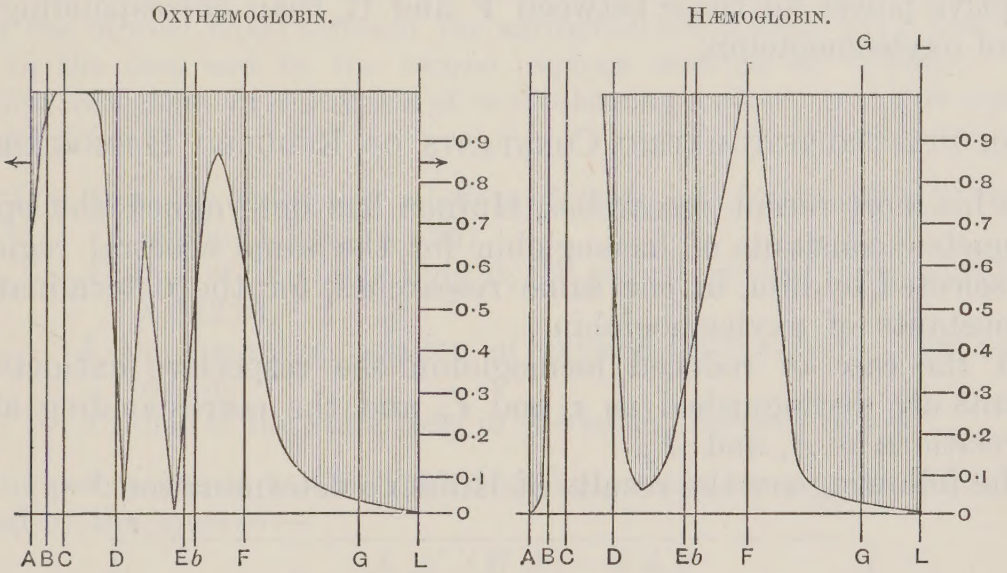

Fig. 34.—Graphic representation of the spectrum of—(1) oxyhæmoglobin and (2) hæmoglobin. The numbers at the right-hand side of each diagram indicate percentages.—After Rollett.

This dichroism is also characteristic of the blood of asphyxiated animals, and was first observed by Brücke. *It is specially to be noted that, whilst solutions of reduced hæmoglobin are dichroic, solutions of the* O_2- CO- *and* NO-*compounds of hæmoglobin exhibit no trace of dichroism.*

Cause of the differences observed in the colour of blood contrasted with that of solutions of hæmoglobin.—The much brighter colour presented by blood, as contrasted with corresponding solutions of the blood-colouring matter, depends upon the presence of the blood corpuscles. Were we to conceive, as Rollett argues, the blood corpuscles suspended in the liquor sanguinis or in serum, and retaining all their physical properties *save their colour*, then, as a result of the repeated total reflections, due to the differences in the refractive indices of the corpuscles and the fluid in which they float, blood would appear as white as milk.

[1] Hüfner, *op. cit.*, *Arch. f. Physiol.*, Leipzig, 1894, S. 150.

But these total reflections *do go on* in the case of the actual coloured corpuscles in a precisely similar manner to that which would occur in the hypothetical case just discussed; and the light reflected by them is conditioned by, and corresponds to, the absorption of the spectral colours exerted by the hæmoglobin and the oxyhæmoglobin respectively.

The visible spectrum of reduced hæmoglobin.—It has already been stated that, when a solution of oxyhæmoglobin is reduced, the two absorption-bands α and β disappear and are replaced by a single one (γ) situated between D and E, which is less deeply shaded and possesses less sharply-defined edges (see Plate I., Spectrum 5). This summary description must now be supplemented.

The right-hand diagram on p. 233 exhibits fairly accurately the absorption of light by solutions of reduced hæmoglobin of varying concentrations. The single absorption-band (γ), though occupying in solutions of from 0·2 to 0·4 per cent. and 1 cm. in thickness, the greater part of the space between Frauenhofer's lines D and E, has its centre or darkest region rather nearer D than E. According to my own measurements, the darkest part of the band corresponds approximately to λ 550.

It is to be noted that solutions of reduced hæmoglobin have a much greater absorptive power for the rays between A and B, and a smaller absorptive power for those between F and G, than corresponding solutions of oxyhæmoglobin.

The Spectrophotometric Constants of Reduced Hæmoglobin.

In his most recent researches, Hüfner has determined the spectrophotometric constants of hæmoglobin for the same spectral regions as were selected by him, in the same researches, for the determination of the constants of oxyhæmoglobin.

In the case of reduced hæmoglobin the respective extinction-coefficients are distinguished as ε_r and ε'_r, and the corresponding absorption relations as A_r and A'_r.

The following are the results of Hüfner's determination:[1]—

A_r	A'_r
(λ 554–λ 556)	(λ 531·5–λ 542·5)
0·001354	0·001778

The quotient $\frac{\varepsilon'_r}{\varepsilon_r}$ is a constant of special importance; it is 0·7617.

The value of the quotient $\frac{\varepsilon_o}{\varepsilon_r}$ has also been determined by Hüfner;[1] it is 0·6541.

The determination of the amount of oxy- and reduced hæmoglobin when both are present.—Vierordt pointed out that the absorption

[1] G. Hüfner, "Neue Versuche zur Bestimmung der Sauerstoffcapacität des Blutfarbstoffs," *Arch. f. Physiol.*, Leipzig, 1894, S. 140.

of light (as determined by the extinction-coefficient) in a definite spectral region, exerted by a mixture of two or more colouring matters, is the sum of the extinction-coefficients of each of its coloured constituents; and that in the case of a solution containing *two* colouring matters, if we are acquainted with the optical constants of each in *two and the same* spectral regions, we are able by the spectrophotometer to determine the relative and absolute amount of each constituent. In a similar manner we should, according to theory, be able to determine the amounts of three or of x colouring matters coexisting in a solution, if we were acquainted with the value of A in three and the same, or in x and the same spectral regions. The immense importance of a method which permits of the accurate determination of oxy- and reduced hæmoglobin in blood, and which furnishes us with essential data for calculating the amount of oxygen present in combination with hæmoglobin, makes it necessary that we should explain the nature of the very simple calculations which enable us, from the determination of the extinction-coefficients in two spectral regions, to effect a determination which, so far as I know, cannot be carried out with any pretence to scientific accuracy, or even with any claim to be presumably correct, by any other process whatsoever.

We shall assume that, by following methods which we shall not attempt to describe, but for which the reader is referred to Hüfner's original papers, blood has been diluted with 0·1 per cent. of aqueous solution of NaOH, *under conditions which preclude the possibility of contact with oxygen*, and that in the diluted blood solution the extinction-coefficients have been determined in the first and in the second regions selected by Hüfner. These extinction-coefficients of a *mixture* of two colouring matters, we shall represent by E and $E.'$

Let A_r be the absorption relation of (reduced) hæmoglobin in the first region (λ 554 – λ 556).

A'_r that of the same body in the second spectral region (λ 531·5 – λ 542·5).

A_o the absorption relation of oxyhæmoglobin in the first spectral region.

A'_o that of the same body in the second spectral region.

Then the percentage of (reduced) hæmoglobin, which we may designate x, will be found by the equation—

$$x = \frac{A_r A'_r (E' A'_o - E A_o)}{A'_o A_r - A_o A'_r}$$

and the percentage of oxyhæmoglobin by the following equation—

$$y = \frac{A_o A'_o (E A_r - E' A'_r)}{A'_o A_r - A_o A'_r}$$

Having thus determined by spectrophotometry the amount of oxyhæmoglobin by weight existing in a known volume, say 100 c.c. of blood, we can ascertain the volume of the respiratory oxygen measured at 0° C. and 760 mm. pressure (which could, but probably with less accuracy, be likewise determined with the aid of the mercurial pump and subsequent analyses of the gases boiled out of the blood) by multiplying each gramme of oxyhæmoglobin found by 1·338 (or 1·34). In this manner Hüfner, having determined the relative and absolute amounts of hæmoglobin and oxyhæmoglobin in the blood, drawn simultaneously from the main artery and vein of a limb, ascertained the amount of oxygen in each. There is a strong presumption that determinations of oxygen made in this manner are nearer the truth than those which the more complex and laborious methods by means of the mercurial pump and gas analysis are capable of giving. In the process of raising the blood to a temperature of at least 40° C. in the exhausted

chamber connected with the mercurial pump, some of the oxygen must be used up in oxidising the readily oxidisable substances existing in the blood, and especially in venous blood, and an error will be thereby introduced unequally affecting different samples of blood,—an error which is influenced by the duration and extent to which the heat is applied to the blood and the rapidity with which the aqueous vapour and gases evolved by the blood are removed.

The photographic spectrum of reduced hæmoglobin. — When the molecule of *dissociable* oxygen is removed from oxyhæmoglobin, either by the action of reducing agents, or by boiling *in vacuo*, the absorption-band in the extreme violet is remarkably displaced towards the less refrangible end of the spectrum, the centre of absorption corresponding to λ 426·0. The difference in the position of Soret's band in the oxy- and in reduced hæmoglobin is shown in the photo-type (Fig. 33). When we reflect that the addition of a molecule of oxygen to the enormous molecule of hæmoglobin cannot affect in an appreciable manner the mass of the molecule, we must conclude that the displacement of the absorption-band towards the ultra-violet end when hæmoglobin combines with oxygen (all other conditions remaining the same), indicates that this combination leads to a notable acceleration of the motion of the intramolecular group of carbon atoms upon which the extreme violet absorption-band depends.

The amount of oxygen with which hæmoglobin combines to form oxyhæmoglobin.—It is believed, on various grounds, that one molecule of hæmoglobin combines with one molecule of oxygen to form the compound which we know as oxyhæmoglobin.

The most recent determinations made by Hüfner have shown that 1 grm. of reduced hæmoglobin of the ox can link to itself 1·338 c.c. of oxygen or carbonic oxide (measured at 0° C. and 760 mm. pressure). The molecular weight of the hæmoglobin of the ox (calculated from Hüfner's most recent estimations of the iron which this body contains) = 16669. The volume of oxygen absorbed by reduced hæmoglobin, calculated from this molecular weight, should be 1·34 c.c., so that the result of experiment agrees in a surprising manner with theory.

Differences in chemical reactions between solutions of reduced and oxyhæmoglobin.—1. Solutions of reduced hæmoglobin when boiled *in vacuo*, or subjected to the action of CO, unlike solutions of oxyhæmoglobin, yield no oxygen.

2. They are not decomposed even by long contact with trypsin, which readily splits up oxyhæmoglobin into hæmatin and the products of trypsin proteolysis.

3. They are unaffected by H_2S, which, when acting for a sufficient length of time upon oxyhæmoglobin, converts it into sulpho-methæmoglobin.

4. Nitrites, potassium ferricyanide, and permanganate, and many other oxidising and reducing agents, exert no action on reduced hæmoglobin, whilst they convert oxyhæmoglobin into methæmoglobin.

5. When treated with alcoholic or watery solutions of acids or alkalies, in the complete absence of free oxygen, hæmoglobin yields purple-red solutions or precipitates. The hæmoglobin is, under these circumstances, split up into an iron-containing coloured body—*hæmochromogen*—and into an albuminous body or bodies. Oxyhæmoglobin, under the same conditions, splits up into an iron-containing body—*hæmatin*—and albuminous products.

Non-existence of the so-called "pseudo-hæmoglobin."—After treating blood with reducing agents until the two bands of oxyhæmoglobin were no longer visible, Siegfried[1] found that there yet remained oxygen removable by boiling in a barometric vacuum. He therefore concluded that, in addition to oxyhæmoglobin, there existed another oxygen compound of hæmoglobin, and that this is characterised by the same absorption spectrum as reduced hæmoglobin. To this hypothetical body he gave the name of *pseudo-hæmoglobin.* Its existence has been *absolutely disproved* by Hüfner.[2] The mistake into which Siegfried fell illustrates the danger of drawing conclusions from qualitative spectroscopic observations. Hüfner has shown that without spectrophotometric determinations it is impossible to know whether a solution of blood or of hæmoglobin is completely reduced. The only reliable criterion is to be obtained by determining the values of ϵ_r and ϵ'_r so as to ascertain the quotient $\frac{\epsilon'_r}{\epsilon_r}$ which should = 0·7617.

Blood which has been proved to be completely reduced in this manner, yields no trace of oxygen when boiled in a mercurial pump.

The Compounds of Hæmoglobin with Carbonic Oxide and Nitric Oxide, and their relation to Oxyhæmoglobin.

Introductory remarks.—In a previous part of this article, I have referred to oxyhæmoglobin as an easily dissociated compound, formed by the linking of one molecule of oxygen to a molecule of the highly complex, iron-containing, crystalline colouring matter, "hæmoglobin," and I have subsequently shown that this conception has received confirmation through the fine researches of Hüfner on the molecular weight of hæmoglobin and on the volume of oxygen with which it can combine. In the present section, reference must be made to additional facts which, besides possessing an interest of their own, throw fresh light on the nature of oxyhæmoglobin, and, in a measure, on the function subserved by it, although this subject will be more fully discussed under the heading of "Respiration."

It had been noticed independently by Claude Bernard[3] and by Hoppe,[4] that blood which had been treated with carbonic oxide, or the blood of men and animals asphyxiated by charcoal fumes, presents an intensely bright arterial colour, but that, unlike arterial blood, it does not in a few hours change to a venous hue, but retains its vermilion tint for long periods of time. The idea forced itself on the minds of both Claude Bernard and Hoppe, that through the action of CO the power which the coloured corpuscles possess of acting as oxygen-carriers had in some way been interfered with. Claude Bernard has, however, the merit of being the first to show that, when brought in contact with the blood, CO is absorbed and displaces oxygen; and he afterwards based upon these facts a method for the quantitative determination of the oxygen of the blood.

At the same time as, and independently of, Bernard, Lothar Meyer[5]

[1] "Ueber Hämoglobin," *Arch. f. Physiol.*, Leipzig, 1890, Phys. Abth., S. 385.

[2] "Bestimmung der Sauerstoffcapacität des Blutfarbstoffs," *ibid.*, 1894, Phys. Abth., S. 140 and 175.

[3] "Leçons sur les effets des substances toxiques et médicamenteuses," Paris, 1857, p. 158; also "Propriétés des liq. de l'organisme," Paris, 1859, tome i. p. 355.

[4] "Ueber die Einwirkung des Kohlenoxydgases auf das Hämatoglobulin," *Virchow's Archiv*, 1857, Bd. xi. S. 288.

[5] "Die Gase des Blutes," Göttingen, 1857; and *Ztschr. f. rat. Med.*, 1858, S. 256; "De sanguine oxydo carbonico infecto," Diss. Inaug., *Vratislaviæ*, 1858.

showed—(1) that the absorption of oxygen and carbonic oxide by blood does not proceed according to Dalton and Henry's law—a proof, amongst many others, that these gases are chemically combined with some constituent of the blood and not held in a state of simple solution; (2) that blood which has been deprived of its gases by boiling *in vacuo*, combines with the same volume of carbonic oxide as of oxygen—in other words, that when carbonic oxide replaces the oxygen of the blood, one molecule of the former takes the place of one molecule of the latter (*i.e.* CO replaces O_2). Lothar Meyer further showed that hæmatin could *not* be the body with which O_2 and CO entered into combination, and expressed the surmise that it might prove to be the same as constituted the red blood crystals described by Lehmann.[1] The truth of the surmise was soon proved beyond the possibility of doubt, it being shown that the O_2- and CO-compounds of the blood-colouring matter are isomorphous, that they are characterised by a similarity in their power of absorbing light, but that the CO-compound is distinguished by not being decomposed by reducing agents (Hoppe-Seyler).

Hermann[2] subsequently showed that just as CO possesses the power of displacing the oxygen of oxyhæmoglobin, nitric oxide (NO) in its turn is capable of displacing CO, one molecule of the former replacing one molecule of the latter, the NO-compound being, like the CO-compound, absolutely irreducible.

The three compounds of hæmoglobin were shown to be isomorphous, to be characterised by a highly florid colour, only slightly differing in tint one from the other; their visible spectrum was found to be distinguished by two absorption-bands between D and E, at first sight appearing identical in the three cases, though careful measurement revealed a very slight shifting of the bands towards the more refrangible end of the spectrum in the case of the CO-compound.

They were all three found to be free from pleochromatism—a character in which they differ strikingly from reduced hæmoglobin. Whilst the CO-compound is much more stable than the O_2-compound, the NO-compound is again more stable than the CO-compound.

It was at first believed that the CO-compound, unlike oxyhæmoglobin, could not be dissociated. I was the first to show that by the long-continued passage of neutral gases through solutions of CO-hæmoglobin, the CO is gradually driven out, and reduced hæmoglobin is obtained.[3] Donders,[4] to whom the discovery of the fact is always ascribed, drew attention to it in a highly interesting theoretical paper. Zuntz[5] immediately afterwards showed, in contradiction of Nawrocki,[6] that blood saturated with carbonic oxide, when boiled *in vacuo*, gives up its carbonic oxide and that it manifests the absorption-band of reduced

[1] "Consideranti enim quæ his in rebus din versatus Lehmann de rubris illis sanguinis crystallis nuper publicavit, plus quam verisimile videbitur, hac cum substantia et oxygenium et oxydum carbonicum conjunctionem chymicam posse inire"; Lothar Meyer, "De sanguine oxydo carbonico infecto," p. 12.

[2] "Ueber die Wirkungen des Stickstoffoxydgases auf das Blut," *Arch. f. Physiol.*, Leipzig, 1865, S. 469.

[3] A. Gamgee, "On Poisoning by Carbonic Oxide Gas, and by Charcoal Fumes," *Journ. Anat. and Physiol.*, London, 1867, vol. i. pp. 339–346.

[4] Donders, "Der Chemismus der Athmung, ein Dissociations-process," *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 20–26.

[5] "Ist Kohlenoxydhämoglobin eine feste Verbindung?" *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 584–588.

[6] "De Claudii Bernardi methodo oxygenii copiam in sanguine determinandi," Inaug. Diss., *Vratislaviæ*, 1863.

hæmoglobin, and Podolinski[1] succeeded in dissociating blood saturated with nitric oxide, by passing a stream of hydrogen through it for an hour and a half; at the end of which time the blood presented the absorption-band of reduced hæmoglobin.

Having passed in review the chief facts which exhibit the relationship existing between the different compounds of hæmoglobin, and which illustrate the nature of the combination of hæmoglobin with gases, some of the characters and properties of CO-hæmoglobin and NO-hæmoglobin, but particularly of the former, must be systematically though briefly described.

Carbonic Oxide Hæmoglobin (CO-Hæmoglobin).

Mode of preparation.—A current of pure carbon monoxide is passed through a saturated solution of oxyhæmoglobin. The solution acquires a carmine-like tint in contrast to the scarlet colour of oxyhæmoglobin. This solution is then cooled to 0° C., and, after being treated with one-fourth of its volume of alcohol previously cooled to 0° C., is set aside at a temperature which must not rise above 0° C., but which should be as low as possible. After some hours or days, the CO-compound, which is more sparingly soluble than O_2-hæmoglobin, separates in crystals, of which the forms are identical with those of that body.

The absorption of light by CO-hæmoglobin.—(*a*) **The visible spectrum.**—Solutions of this body possess more of a bluish-red tint than the O_2-compound. If solutions of equal concentration of the oxygen and carbonic-oxide compounds be compared, it will be found, on spectroscopic examination, that the CO-compound absorbs the blue rays of the spectrum to a less degree than oxyhæmoglobin.

Between D and E are seen two absorption-bands which, unless very closely studied, appear absolutely identical with those of oxyhæmoglobin (see Plate I., Spectrum 6). On careful measurement, however, it is seen that both the bands are very slightly shifted in the direction of E; that is to say, towards the violet end. This is best seen by noticing the interval between D and the adjacent border of the first absorption-band; in the case of the CO-compound this interval is broader than in that of the O_2-compound.

The spectrophotometric constants of CO-hæmoglobin.—These constants were re-determined by Hüfner in 1894, at the same time as those of oxy- and reduced hæmoglobin, and for the same spectral regions, with the results exhibited below.[2] The coefficients of extinction in the case of CO-hæmoglobin are designated for the region λ 554–λ 565, ϵ_c, and for the region λ 531·5–λ 542·5 ϵ'_c, whilst the corresponding absorptive relations are designated A_c and A'_c.

A_c	A'_c
λ 554–λ 565	λ 531·5–λ 542·5
0·001383	0·001263

[1] "Ueber die Austreibbarkeit des CO- und NO- aus dem Blute," *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 553–555.

[2] Hüfner, *op. cit.*, S. 141 and 142.

(*b*) **The photographic spectrum of CO-hæmoglobin.**—In Fig. 35 are shown reproductions of the photographic spectrum of this compound, contrasted with that of the oxygen compound. The band of Soret is just as well marked in the one as in the other, but in the case of the CO-hæmoglobin there is a decided shifting of the band in the extreme violet towards the red, which is somewhat curious, considering that the bands in the visible spectrum are, *though to a much less extent*, shifted in the opposite direction. I have shown that there is absolute identity in the position of the absorption-band in the extreme violet, in the case of the CO- and NO- compounds of hæmoglobin.[1]

The principal characteristic reactions of CO-hæmoglobin.—1. When treated with Stokes' reagent, solutions of ammonium sulphide, and the like, no change whatever occurs, either in the colour or the spectrum of blood saturated with carbonic oxide, or in solutions of pure CO-hæmoglobin.

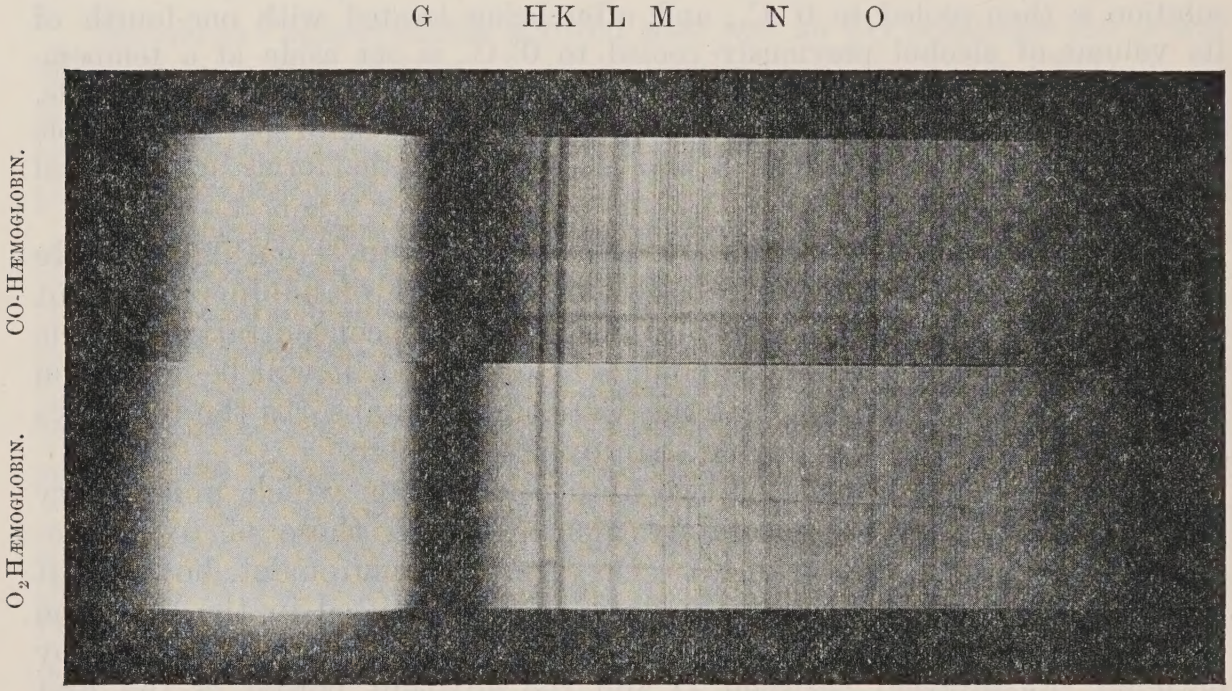

FIG. 35.—The photographic spectrum of oxyhæmoglobin and of CO-hæmoglobin.

2. The blood of men or animals asphyxiated by carbonic oxide, or by a gas containing it (charcoal fumes, coal gas), if pretty fully saturated, possesses and retains for a long time a florid arterial colour, and when diluted is found to be partially or completely irreducible. Hoppe-Seyler found that if such blood is sealed in glass tubes, it may retain for some years its characteristic spectroscopic properties, and even admit of CO being boiled out, with the aid of the mercurial pump, and identified by chemical analysis.

3. The addition of a concentrated solution of sodium hydrate (density 1·3) to blood, saturated with CO in the proportion of about two parts of the former to one of the latter, causes the blood to assume a fine scarlet colour, and to deposit a cinnabar-red precipitate. The same coloration and precipitate is produced with solutions of pure CO-hæmoglobin. According to Hoppe-Seyler, the precipitate is composed of CO-hæmoglobin, rapidly passing into CO-hæmochromogen. When normal blood is treated in the same way with sodium hydrate, it is

[1] Gamgee, *Proc. Roy. Soc. London*, 1896, vol. lix. p. 276.

converted into a black shining mass, which when spread in thin layers over porcelain appears of a greenish-brown colour.

4. Aqueous neutral solutions of pure CO-hæmoglobin, when heated to boiling point, furnish a bright red precipitate, composed of coagulated albuminous substances and CO-hæmochromogen (Hoppe-Seyler).

5. Solutions of carbonic oxide hæmoglobin, treated with NO in the absence of oxygen, are at once decomposed, and liberate CO (Hüfner).

Nitric Oxide Hæmoglobin (NO-Hæmoglobin).

Mode of preparation.—So great is the affinity of nitric oxide for oxygen, that, when it comes in contact with it, deep red fumes of nitrogen peroxide, NO_2, is formed. When this gas comes in contact with water, the decomposition indicated in the following equation occurs :—

$$3NO_2 + H_2O = 2HNO_3 + NO.$$

But as all free acids decompose the colouring matter of the blood, before causing nitric oxide to act upon blood certain precautions must be taken ; for even if atmospheric oxygen be eliminated and nitric oxide caused to act upon oxyhæmoglobin, nitrous oxide would be formed at the expense of the oxygen of that body ; and next, by the action of water, nitric acid, which would immediately decompose the hæmoglobin.

Two methods of proceeding are open to us—(*a*) To add to the solution of oxyhæmoglobin which is to be subjected to the action of nitric oxide, sufficient alkali to neutralise the nitric acid which will be formed. Such a solution must be placed in a flask, permitting of the whole of the air above the solution being driven out and replaced by a neutral gas, before allowing access to the nitric oxide. After the latter has exerted its action, care must be taken again to pass a neutral gas through the apparatus and solution, so as to remove all traces of free nitric oxide.

(*b*) The solution of oxyhæmoglobin is subjected to the long-continued action of carbonic oxide, so as to form CO-hæmoglobin and to expel all traces of dissolved oxygen. Otherwise, the process is constructed as described under (*a*). This process would be certainly preferred, if it were desired to crystallise the NO-compound.

Physical and chemical characters.—Blood saturated with nitric oxide possesses almost as florid a colour as CO-blood, though Hermann says that it does not present the slight bluish shade of the latter. It exhibits no dichroism. Solutions of NO-hæmoglobin, or diluted NO-blood, exhibit a visible spectrum in which, as I have convinced myself, the bands occupy *precisely* the position of the two oxyhæmoglobin bands. In the photographic spectrum, however, the band in the extreme violet exhibits *absolute coincidence* with that of CO-hæmoglobin.

NO-hæmoglobin can be crystallised, and, as Hermann showed, the crystals are identical with those of oxyhæmoglobin and CO-hæmoglobin.

Alleged (but Problematical) Compounds of Hæmoglobin with Gases.

1. With hydrocyanic acid.—The most discrepant statements have been made in reference to the very simple question—whether hydrocyanic acid added to, or passed through, blood affects the characters of its absorption-spectrum. In spite of these, it may be definitely stated that, at ordinary temperatures, and when acting for moderate periods, hydrocyanic acid leads

to no change in the physical characters of the blood, of which the spectrum remains unchanged, and of which the property of being reduced by suitable agents remains unaffected.

Upon what appears to me to be altogether insufficient evidence, Hoppe-Seyler,[1] however, came to the conclusion that hydrocyanic acid forms an easily decomposed compound with hæmoglobin. If hydrocyanic acid be added to a solution of oxyhæmoglobin, on crystallising out the latter it retains some of the acid. These crystals may be repeatedly crystallised, and when dried *in vacuo* over sulphuric acid they are found to contain hydrocyanic acid. The supposed compound of hydrocyanic acid with oxyhæmoglobin presents an absorption-spectrum absolutely identical with that of oxyhæmoglobin, and is reduced just as easily by such agents as ammonium sulphide or Stokes's reagent. On the other hand, blood to which hydrocyanic acid has been added shows the bands of oxyhæmoglobin for a much longer time than normal blood.

It appears to me that no proof whatever has been advanced of the existence of a chemical compound of oxyhæmoglobin with HCN.

That some hydrocyanic acid should adhere to hæmoglobin, as it crystallises out of the mother liquor which contains the acid, is quite in accordance with a number of experiences of a similar kind, and can by itself afford no evidence of an actual compound existing. The resistance of blood to which hydrocyanic acid has been added, to decomposition, when confined in a sealed or closed vessel, can, on the other hand, be easily explained by the unquestionable arrest or slowing of the process of putrefaction in the presence of hydrocyanic acid. It is, undoubtedly, the products of putrefaction which are the causes of the *apparently* spontaneous reduction of the oxyhæmoglobin of blood confined in a receptacle to which air has no access; so that an agent which does inhibit putrefaction—as hydrocyanic acid unquestionably and admittedly does—and, at the same time, does not, at ordinary temperatures, decompose oxyhæmoglobin, would be expected to act as hydrocyanic acid has been found to do in furthering the persistence of the oxyhæmoglobin bands.

What I have just stated in reference to the probable non-existence of a compound of HCN with oxyhæmoglobin, does not imply my disbelief in the existence of an interesting compound of hydrocyanic acid with methæmoglobin, described by Kobert, which will be discussed after the latter body has been described.

2. With cyanogen.—Ray Lankester[2] believed that cyanogen formed a compound with hæmoglobin, probably analogous to the CO- and NO-compounds, and characterised by an absorption-band, resembling that of, but obviously not due to, reduced hæmoglobin. Many discordant statements have been published on this matter. It appears that by the prolonged action of cyanogen, as by the prolonged action of HCN, there is produced Kobert's cyanogenmethæmoglobin (see p. 248).

3. With acetylene (C_2H_2).—Bistrow and Liebreich[3] surmised that acetylene forms a very unstable compound with hæmoglobin, easily reducible by sulphide of ammonium and similar agents. On the evidence at present at our disposal, the existence of this compound must be considered as more than problematical.

4. With carbon dioxide.—According to Bohr, hæmoglobin forms a series of compounds with carbon dioxide, which possess spectra identical with those of reduced hæmoglobin. He states, further, that if a solution of hæmoglobin be brought in contact with a mixture of oxygen and carbon dioxide, the

[1] "Cyanwasserstoffhæmoglobinverbindungen," *Med.-chem. Untersuch.*, Berlin, 1868, S. 206–208.

[2] "Ueber den Einfluss des Cyangases auf Hämoglobin nach spectroscopischen Beobachtungen," *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 491–493.

[3] "Ueber die Wirkung des Acetylens auf das Blut," *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1868, Bd. i. S. 220.

amount of either of these gases which is absorbed is independent of the other.

A careful study of the whole of Bohr's researches on this subject, as well as those on the various hypothetical compounds of hæmoglobin with oxygen, has convinced me that his work is pervaded by fallacies, which spring in part from erroneous methods of work, in part from a non-appreciation of physical principles of which the exactitude is beyond dispute; the discussion of Bohr's statements in a text-book would be, under these circumstances, altogether out of the question.

THE IMMEDIATE DERIVATIVES AND PRODUCTS OF DECOMPOSITION OF OXYHÆMOGLOBIN AND REDUCED HÆMOGLOBIN.

Introductory observations.—It has already been stated, that when the blood-colouring matter is subjected to the action of strong alkalies and of acids, or even of salts possessing an acid reaction, or to the action of heat, of alcohol, and of many other chemical agents, it undergoes a decomposition of which the chief products are an albuminous substance or substances, and a colouring matter which contains the whole of the iron originally present in the oxyhæmoglobin or hæmoglobin decomposed.

Under ordinary circumstances, when oxyhæmoglobin is decomposed in the presence of air, the coloured product of decomposition is the body we know as *hæmatin*, the amount of which produced corresponds theoretically to 3·8 per cent. of the oxyhæmoglobin. Traces of organic acids are said to result from the decomposition, the main product of which is, however, composed of the albuminous residue of the blood-colouring matter (*vide infra*). If, however, instead of decomposing oxyhæmoglobin, we employ reduced hæmoglobin and carry out the process in the complete absence of oxygen, we obtain, not hæmatin, but a body of which some of the optical characters were first described by Stokes, and which he named *reduced hæmatin*, to indicate that it may be obtained by the action of reducing agents on hæmatin. Instead of employing this term, it is better to adopt that of hæmochromogen, introduced by Hoppe-Seyler, to whom we owe nearly all the knowledge we possess with regard to it. According to Hoppe-Seyler, hæmochromogen constitutes the coloured radicle of the blood-colouring matter, upon which its essential optical properties and its property of combining with oxygen, carbonic oxide, and nitric oxide depend.

Under the influence of carbonic acid, and very dilute acids acting for comparatively short periods of time, oxyhæmoglobin, long before the complete splitting up into hæmatin, undergoes a change which is doubtless of the nature of a decomposition; this change is identical with that which is also brought about by a variety of oxidising agents, typically by ozone, nitrites, and potassium ferricyanide; to the body which results, the name of methæmoglobin has been given. It will be considered first amongst the decomposition products of oxyhæmoglobin. We shall show it to be a substance which is formed in the living body, under the influence of certain poisonous agents, and is occasionally found in old blood extravasations; it possesses the power of forming molecular compounds with certain bodies, such as nitrites, hydrocyanic acid, and cyanogen.

The Albuminous Residue of the Blood-Colouring Matter.

An unfortunate error has become popular, and has, indeed, been propagated by a large number of text-books, namely, that when oxyhæmoglobin is decomposed, it splits up into hæmatin and a definite albuminous matter belonging to the group of globulins, and designated *globin*. There is absolutely no ground for such a statement. The term *globin* was, it is true, assigned by Preyer to an albuminous substance, which he obtained as a product of the spontaneous decomposition of solutions of oxyhæmoglobin, but this body did not possess the characteristic properties of the globulins, and there is no ground for considering it as representing the albuminous body which, by linking to itself a coloured iron-containing radicle, forms crystalline hæmoglobin.

Our knowledge on this matter is indeed of the most unsatisfactory character. We know, and have shown (see p. 207), that solutions of oxyhæmoglobin in the presence of many of the reagents for albumin (so long as these do not decompose the blood-colouring matter) behave quite differently from solutions of the native albumins, globulins, etc. Thus copper sulphate, mercuric chloride, silver nitrate, and the acetates of lead do not produce even a cloudiness when added to solutions of *pure* hæmoglobin, so long as this remains undecomposed. It has long been recognised, too, that Lehmann's hypothesis, that the blood-colouring matter was composed of colourless crystals tinted by a red pigment, was false; but as to the true nature of the albuminous residue, we have very little knowledge, though the facts in our possession almost force us to the conclusion that it is not identical in all animals, as shown by the difference in the percentage of sulphur in the hæmoglobin of the horse and the dog.

The reagents which we employ to decompose the blood-colouring matter yield us derivatives of the albuminous residue, not the body itself; we obtain acid albumin as a result of treatment with acids, alkaline albuminates as a result of treatment with alkalies. The most interesting observations on the albuminous products of the decomposition of oxyhæmoglobin were published by Kühne[1] thirty years ago. He showed that when CO_2 is passed through solutions of pure oxyhæmoglobin a flocculent precipitate is thrown down, which *does not possess*, as had been erroneously asserted by A. Schmidt, *fibrinoplastic properties*, and which *does not behave as a globulin*. According to Kühne, this precipitate possesses so peculiar an appearance under the microscope that it cannot be mistaken for any other substance. It forms long colourless fibres which are so like fibres of connective tissue that they might be taken for them. This substance differs fundamentally from globulin; it is, for example, insoluble in water containing oxygen in solution.

Methæmoglobin.

Hoppe-Seyler was the first[2] to observe that solutions of oxyhæmoglobin exposed to the air, or filter papers saturated with such solutions, often assume a brown colour. Under these circumstances, the solution

[1] "Lehrbuch," 1866, S. 206, 207.

[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1864, No. 53. See also *Med.-chem. Untersuch.*, Berlin, S. 378, und "Die Zusämmensetzung des Methämoglobin, und seine Umwandlung zu Oxyhämoglobin," *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 150, 155.

is found to have become acid and to exhibit a spectrum in which, in addition to the two bands of oxyhæmoglobin, one is seen in the red, occupying much the position of the band of acid hematin.

Hoppe-Seyler applied the name of methæmoglobin to the very indefinite and problematical body whose solutions possessed the above characters, and held it to be a product of the partial reduction of oxyhæmoglobin, derived from it by the removal of a portion of the dissociable oxygen of that compound.

I myself, soon after, investigated the changes brought about in the properties of oxyhæmoglobin under the influence of *nitrites*, and in a memoir,[1] of which the experimental facts have, so far as they have yet been controlled, been confirmed in every particular, pointed out the remarkable phenomena which attended the conversion of oxyhæmoglobin into methæmoglobin, though I committed the error of believing that the changes described by me were due to the combination of nitrites with oxyhæmoglobin, and not to an action which was afterwards shown to be possessed by a large number of both oxidising and reducing substances. I showed that blood which had been acted upon by nitrites, in addition to marked and definite changes in colour and spectrum, had almost entirely lost its power of absorbing oxygen from the atmosphere; that, under the influence of nitrites, the oxygen of oxyhæmoglobin *is not removed*, but passes into a condition in which *it is no longer removable* by boiling *in vacuo* or by the action of carbonic oxide. The action of reducing agents reveals, however, as I showed, that the molecule of loose oxygen of oxyhæmoglobin is still present in blood which has been acted upon by nitrites, for, in the absence of all traces of oxygen, reducing agents first of all and instantaneously liberate oxyhæmoglobin, which is only afterwards reduced. I pointed out that the chocolate-coloured nitrite blood can be crystallised, the colouring matter being isomorphous with hæmoglobin and its compounds, and that the crystals contain the nitrite which has brought about the change, though I showed that the composition of these *molecular compounds* of oxyhæmoglobin is not a constant one. After innumerable contradictions, it has been proved, though without a word of acknowledgment, mainly by the researches of Hüfner and his pupils, that my account of the changes which characterise the formation of methæmoglobin was, in every particular, exact, whilst the comparatively recent statement, by Kobert, of the existence of combinations of hydrocyanic acid and cyanides with methæmoglobin is an illustration of the class of compounds of oxyhæmoglobin which I was the first to discover and describe, and of which doubtless a large number will be obtained.

Mode of preparation.—A large number of inorganic and organic bodies, acting upon solutions of oxyhæmoglobin, convert it into methæmoglobin. The chief of these are potassium ferricyanide—which, on account of the rapidity of its action, is to be preferred to all others—nitrites, chlorates, potassium permanganate, nitrobenzol, pyrogallol, pyrocatechin, acetanilid, etc.

In order to study the spectroscopic characters of methæmoglobin, a solution of diluted blood is treated with a few drops of a strong solution of potassium ferricyanide, when the change in colour and spectrum is seen to occur almost instantly. To prepare the crystalline colouring matter, 2 or 3 c.c. of a saturated solution of potassium ferricyanide or of a nitrite is

[1] A. Gamgee, "On the Action of Nitrites on Blood," *Phil. Trans.*, London, 1868, vol. clviii. pp. 589–626.

added to a litre of saturated aqueous solution of crystals of oxyhæmoglobin, and after the conversion into methæmoglobin has occurred, about 25 per cent. of alcohol added. The mixture is then exposed to a temperature below 0° C. I succeeded in recrystallising methæmoglobin prepared by the action of potassium nitrite and of ethyl and amyl nitrites on oxyhæmoglobin.

Chemical and physical characters.—Crystals of methæmoglobin are more sparingly soluble than those of oxyhæmoglobin, and the colorific intensity of their solutions is less.

It is to be noted that, whilst solutions of reduced and oxyhæmoglobin are not precipitated by either neutral or basic lead acetates, these reagents added cautiously, with careful avoidance of an excess, precipitate *methæmoglobin*, *hæmatin*, and *hæmatoporphyrin*, and may be employed for the separation and detection of traces of oxyhæmoglobin when mixed with and concealed by any of the above-mentioned bodies.

Solutions of methæmoglobin, when of a neutral or a slightly acid reaction, possess a chocolate-brown colour. When the solution is rendered alkaline, its colour changes to red without a tinge of the chocolate-brown.

The acid solution is found to present a spectrum in which the oxyhæmoglobin bands α and β are very weak or even not visible, whilst an absorption-band is seen in the red between C and D, and nearer the former. This band occupies nearly, though by no means exactly, the position of a similar band in the spectrum of acid hæmatin (see Plate II., Spectrum 5).

On now rendering the solution alkaline by means of ammonia, the band in the red disappears, and is replaced by a faint absorption-band immediately on the red side of D. By changing the reaction of the solution, the alterations in its colour and spectrum may be repeated indefinitely (Gamgee).

If a solution of methæmoglobin be placed in a deep test tube, in front of a spectroscope, and arrangements be made for allowing a stream of solution of ammonium sulphide to flow to the bottom of the liquid, it can be readily shown that at the very moment of the contact of the reducing and the methæmoglobin solution, the spectrum of oxyhæmoglobin appears; to be subsequently and much more slowly replaced by that of reduced hæmoglobin, which in its turn, when shaken with air, yields oxyhæmoglobin.

A study of the photographic spectrum of methæmoglobin has led me to results of great interest. The conversion of oxyhæmoglobin into methæmoglobin is attended by a shifting of the band of Soret from the extreme violet to the ultra-violet properly so called (Fig. 36). The most persistent part of the band in very dilute solutions, coincides, indeed, with the H and K bands, but the band extends more and more into the ultra-violet, as the concentration of the solution increases. The position and characters of this band in the case of methæmoglobin absolutely corresponds with those of the acid compounds of hæmatin, and not with those presented by hæmoglobin and its compounds, or by hæmochromogen (see Fig. 38).

This spectroscopic character certainly seems to lend weight to the evidence of other kinds, which indicates that methæmoglobin is a first product of the *decomposition* of the oxyhæmoglobin molecule, and that this is a decomposition which leads to the separation of a compound of hæmatin, and not of hæmochromogen. Hoppe-Seyler, indeed, expressed

the opinion that the colouring matter in methæmoglobin is in the same state as in hæmatin, the iron being, as he thought, in the condition of a ferric compound, whilst in oxyhæmoglobin and in hæmochromogen he believed it to exist in a ferrous state, though the grounds for these very definite statements are certainly wanting.

The researches of Hüfner on the oxygen of methæmoglobin. —I had shown that the action of methæmoglobin, as produced by the action of nitrites, could not be attended by a profound alteration in the constitution of oxyhæmoglobin, seeing that the addition of certain reagents at once caused all the effects of the action to disappear, and revealed the continued existence of oxidised hæmoglobin. Nitrites (for these we should now read all agents capable of transforming oxyhæmoglobin into methæmoglobin) had, by my experiments, been shown to resemble in no way those agents which thrust oxygen out of the blood; on the other hand, I had shown that the action of

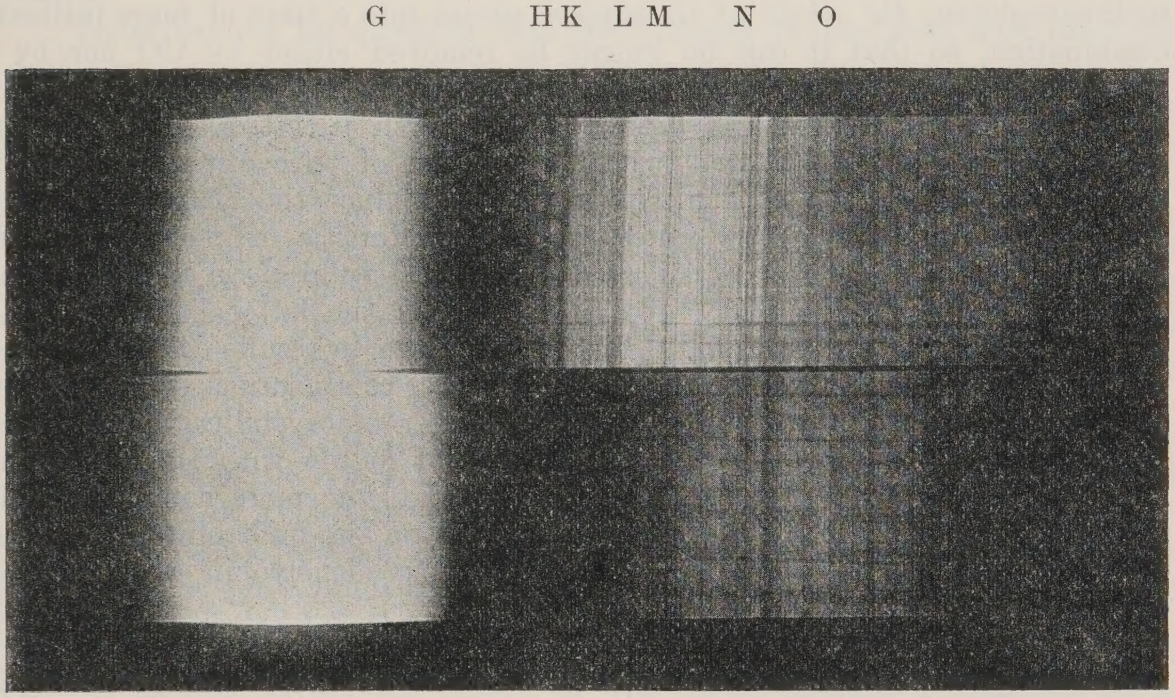

Fig. 36.—The photographic spectrum of oxyhæmoglobin and methæmoglobin.

nitrites resulted in the locking up of the oxygen of the blood, so as to render it irremovable by carbonic oxide, or by a vacuum. But although I had discovered that methæmoglobin, when treated with reducing agents, at once liberates oxyhæmoglobin, I had not been able to show that when the latter substance is converted into the former the whole of its oxygen is locked up without loss, and may be subsequently liberated. This was reserved for Hüfner.

When nitric oxide acts upon a solution of methæmoglobin, the brown colour is changed to bright red, the spectrum of the red solution being identical with that of NO-hæmoglobin. Reflecting on this experiment, Hüfner thought that perhaps NO possesses the power of becoming oxidised to NO_2, at the expense of the oxygen locked up in methæmoglobin (*i.e.* oxygen of the original oxyhæmoglobin which had passed into a more stable combination).

As such might be the case, it occurred to Hüfner to determine the volume of NO_2 produced (for this would bear a definite relation to the O abstracted from methæmoglobin), by causing the nitrous acid (HNO_2), which would be produced by the action of the water of the blood on NO_2, to decompose urea, the N liberated being a measure of the oxygen derived from methæmo-

globin. The ingenious conception of Hüfner will be rendered evident by the three following equations :—

$$(1)\ 6NO + 2(Hb\text{-}O_2) = 4NO_2 + 2(Hb\text{-}NO).$$
$$(2)\ 4(NO_2) + 2(H_2O) = 2(NO_2H) + 2(NO_3H).$$
$$(3)\ 2(NO_2H) + CH_4N_2O = 3(H_2O) + 2CO_2 + 2(N_2).$$

From these equations it results that each molecule of nitrogen liberated will correspond to a molecule of oxygen which had become fixed in methæmoglobin.

Whether the more firmly combined oxygen of methæmoglobin were capable of oxidising nitric oxide or not, the oxygen of oxyhæmoglobin would certainly be able to do so, and Hüfner proceeded to compare the amount of N liberated in the above reaction by solutions of exactly corresponding concentration of oxyhæmoglobin and of alkaline methæmoglobin. The results left no room for doubt, and led to the conclusion that when oxyhæmoglobin is converted into methæmoglobin, *the whole of its oxygen* passes into a state of more intimate combination, so that it can no longer be removed either by CO nor by a vacuum, but is yet available to oxidise such bodies as NO_2.

The compounds of methæmoglobin with nitrites.—I showed, as has already been stated, that when a solution of pure oxyhæmoglobin is treated with a solution of a nitrite, so as to produce the change in colour and spectrum which we now know to be characteristic of methæmoglobin, the blood-colouring matter crystallised out of the solution is found to contain the nitrite, though the proportion in which the latter combines with the hæmoglobin is not constant. The discordance in results did not appear to me surprising, and that "as in the case of other combinations of a molecular kind, such as the union of salts with their water of crystallisation, of bases with sugar, of albumin with metallic oxides, of iodine with the compound ammonias, the amount of the simpler body added to the more complex should vary within wide limits." I further speculated on the probability of a large number of similar combinations to that of oxyhæmoglobin with nitrites existing.

The compounds of methæmoglobin with HCN and cyanides.—It has long been noticed that hypostatic marks on the bodies of men and animals poisoned by prussic acid or metallic cyanides, as well as the mucous membrane of the stomach, present a striking bright red colour. Kobert[1] surmised that this coloration might be due to combination of methæmoglobin with HCN or metallic cyanides, a hypothesis of which he thinks he has obtained confirmation from his experiments. Kobert found that on adding solutions of HCN of *extreme* dilution to a 1 or 2 per cent. solution of methæmoglobin, these assume a beautiful bright red colour, whilst the absorption band or bands of methæmoglobin have disappeared, and are replaced by a single broad absorption-band between D and E, occupying about the position of the band of reduced hæmoglobin. This band cannot, however, be made to disappear by the action of oxygen.

According to Kobert, this band is not affected by the addition of ammonium sulphide. He believes the body which is produced by the action of HCN on methæmoglobin to be a compound of the two bodies, and he ascribes to it the name "cyanogenmethæmoglobin," and represents it for brevity by the symbol CNH·MetHb. He further lays claim to have discovered for the first time similar compounds with nitrites (! !). But Kobert's view of the nature of the action of HCN on methæmoglobin has not been universally accepted. The absorption-spectrum which he has described as characteristic of his new compound is identical with that described by Preyer as resulting from the

[1] "Ueber Cyanmethämoglobin und den Nachweis der Blausäure," Stuttgart, 1891.

action of HCN on oxyhæmoglobin, and by Nawrocki and Lankester as produced when KCN acts upon blood, especially with the aid of gentle heat, and which has generally been held to be a compound of cyanogen and hæmatin (cyanhæmatin).

Szigeti[1] maintains that Kobert's cyanogenmethæmoglobin is in reality cyanhæmatin, the first step in the action of HCN being to split up the methæmoglobin molecule into hæmatin and an albuminous substance. I do not, however, take this view, and, in spite of the evidence which Kobert has adduced being in many respects incomplete, I am inclined to think that the view which he has advanced is correct. In the first place, the certain existence of compounds of the nitrites with methæmoglobin affords presumptive evidence of the strongest kind that similar compounds with such bodies as cyanogen, hydrocyanic acid, and cyanides exist; in the second, the almost instantaneous action of solutions of hydrocyanic acid *of phenomenal dilution* renders it highly improbable that the action of hydrocyanic acid on methæmoglobin is one in which decomposition into hæmatin is a preliminary stage.

There can be no question that HCN acting in the cold, and, for a short time upon, blood or on solutions of oxyhæmoglobin, produces no change in the spectrum, and it is against all experience and analogy from the action of other dilute acids on either oxyhæmoglobin or methæmoglobin to conclude that solutions of HCN of extraordinary dilution should be able—and almost instantaneously—to split up the oxyorthomethæmoglobin molecule. Kobert has found that a solution containing 0·000003 grm. of HCN is able to produce the characteristic change in 1 c.c. of a 1 per cent. solution of methæmoglobin. He has further shown that his assumed cyanogenmethæmoglobin contains HCN which can be recovered from it without loss by distilling with sulphuric acid.

CO-methæmoglobin.—According to Weyl and v. Anrep,[2] this compound is produced when aqueous solutions of iodine and potassium iodide, or solutions of potassium permanganate, continue to act upon a solution of CO–hæmoglobin for several days. This body is said to retain the red colour of CO–hæmoglobin, and to present the same absorption-bands in its spectrum. I fail to understand the grounds for believing in its existence.

Sulpho-methæmoglobin.—This hypothetical body was believed by Hoppe-Seyler[3] to be the cause of the green coloration observed on the surface of putrefying organs.

Sulphuretted hydrogen has no action on reduced hæmoglobin. When acting in small quantities on neutral solutions of pure oxyhæmoglobin, it reduces these. If, simultaneously, a stream of sulphuretted hydrogen and oxygen be passed through blood or neutral solutions of pure oxyhæmoglobin, the solution assumes a green colour in thin, and a red colour in thick layers, and becomes turbid. These solutions are characterised by the presence of two absorption-bands in the red, one on the red side of, but quite close to, C; the other is about midway between C and D, the two bands being united together by a shadow.

It appears to me that there is not the slightest ground for believing that the phenomena above described are due to a definite body,—"sulpho-

[1] "Ueber Cyanhämatin," *Vrtljschr. f. gerichlt. u. öff. Med.*, Berlin, Supp. Bd. vi. S. 9–35. I only know this paper from the abstract by Andreasch in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1883, Bd. xxiii. S. 620.

[2] "Ueber Kohlenoxyd-Hämoglobin," 1. Oxydation von CO–Hb zu Meth–Hb, *Arch. f. Physiol.*, Leipzig, 1880, S. 227–240.

[3] *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, No. 28; "Ueber die Einwirkung des Schwefelwasserstoffs auf d. Blutfarbstoff," *Med.-chem. Untersuch.*, Berlin, S. 651; Araki, "Schwefelmethæmoglobin," *Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xi. S. 412–416.

methæmoglobin"; they are almost certainly caused by a mixture of decomposition products of oxyhæmoglobin, brought about by the action of H_2S upon it.

Hæmatin ($C_{34}H_{35}N_4FeO_5$, Hoppe-Seyler); ($C_{32}H_{30}N_4FeO_3$, Nencki and Sieber).

As has been already stated, hæmatin is the colouring matter which results from the decomposition of oxyhæmoglobin by acids and alkalies. In acid and alkaline solutions the body is characterised by certain spectroscopic appearances, and especially by yielding, *under suitable conditions*, when treated with reducing agents, a body possessing the optical characters, when examined with the spectroscope, which were originally described by Stokes as those of "*reduced hæmatin*," now known as "*hæmochromogen*" (Hoppe-Seyler).

Mode of preparation.—As we are now in possession of an easy and in all respects admirable method of preparing, in a state of great purity, the crystalline hydrochlorate of hæmatin or "hæmin" (see p. 252), the latter body should invariably be employed in the preparation of pure hæmatin.

Pure crystallised hæmin (prepared by Schalfijew's process) is dissolved in a highly dilute solution of potassium hydrate, and the alkaline solution is precipitated by means of dilute hydrochloric acid. The flocculent-brown precipitate is washed with hot distilled water until the washings give no turbidity with silver nitrate. The hæmatin thus precipitated is first dried at the temperature of 100°, and then at 115°, or even higher.

Physical and chemical properties.—Hæmatin has not hitherto been crystallised. In the condition of utmost purity it possesses a bluish-black colour, and a very pronounced metallic lustre. When finely powdered it appears as a dark brown powder, which is distinctly pleochromatic.

It is insoluble in water, alcohol, ether, and chloroform, but slightly soluble in glacial acetic acid; also in acidulated alcohol, but absolutely insoluble in aqueous solutions of acids. It is very readily soluble in all, even highly dilute, alkaline solutions.

Hæmatin forms a crystalline compound with hydrochloric acid (hæmatin hydrochloride, or hæmin), which, because of its importance, will be separately described, and also others with hydrochloric and hydrobromic acids.[1]

It combines with potassium and sodium, as well as with calcium, barium, and other metals. The calcium and barium compounds are obtained by precipitating ammoniacal solutions of hæmatin by means of solutions of calcium or barium chloride, but they have not been yet obtained in a state of purity, and have not been analysed.

Hæmatin may be strongly heated to 180° C. without undergoing decomposition. When heated further it is carbonised without previously melting or taking fire, and liberates hydrocyanic acid, leaving a residue of pure oxide of iron, which amounts to 12·6 per cent. of the hæmatin incinerated.

When boiled with concentrated potassium hydrate, hæmatin undergoes no perceptible change; when fused with caustic potash, it is very slowly decomposed, and evolves ammonia. It is only attacked by

[1] M. C. Husson, *Compt. rend. Acad. d. sc.*, Paris, tome lxxxi. p. 477; V. D. Harris, *Journ. Physiol.*, Cambridge and London, 1885, vol. v. p. 209; D. Axenfield, *Centralbl. f. d. med. Wissensch.*, Berlin, 1885, No. 47.

concentrated hydrochloric acid, at a temperature above 150° C. Concentrated sulphuric acid dissolves it, without any gas being evolved, giving rise to a dark red solution, from which water precipitates the substance known as "hæmatoporphyrin" (see p. 258), which, as it contains no iron, has been sometimes spoken of as iron-free hæmatin. This body is soluble in alkaline solutions, and both its acids and alkaline solutions exhibit very characteristic absorption-spectra.

Alkaline solutions of hæmatin in thick layers, when examined by transmitted light, appear red, whilst thin layers appear of an olive-green colour. Acid solutions, whatever the thickness of the stratum examined, always appear of a brown colour.

When the spectrum of light transmitted through alkaline and acid solutions of hæmatin is examined by the photographic as well as by the direct method, it is seen that the last rays of the spectrum to be absorbed are the red rays up to B; that the solutions are characterised by a defined absorption-band between C and D, which is shifted towards D in the case of the alkaline, towards C in the case of the acid solutions; that alkaline solutions, even when extremely diluted, effect a general absorption of the whole ultra-violet, violet, etc., rays; that acid solutions, even when very highly diluted, whilst not exerting a general absorption of the ultra-violet, exhibit an absorption-band at the junction of the extreme violet and the ultra-violet, properly so called.

The absorption-bands in the visible spectrum of both alkaline and acid solutions of hæmatin are shown in Plate II., Spectra 2, 4, and 6. The alkaline solutions exhibit one absorption-band between C and D, of which the more refrangible border adjoins D, whilst acid solutions exhibit an absorption-band also between C and D, of which the less refrangible border adjoins C, though the position of the band is somewhat influenced by the particular acid which has been employed. Attention is directed to the fact that the band between C and D in the spectrum of methæmoglobin differs in position from the band in the spectrum of acid as well as from that of alkaline hæmatin. Whilst the absorption-band of the former is close to C and that of the latter close to D, the band of methæmoglobin, in acid solutions, is separated by a marked interval both from C and D, though it is closer to the former than to the latter.

Alkaline solutions of hæmatin *in the presence of certain foreign matters*, when treated with reducing agents, exhibit a spectrum which is apparently identical with that which will be described under "Hæmochromogen," and which was first described by Stokes as the spectrum of *reduced hæmatin*. The band in the red disappears, and two characteristic bands appear in the green (Plate II., Spectrum 3). On now shaking the reduced liquid with air, the two bands first referred to disappear, and are replaced by the original hæmatin band.

This experiment would appear to show that hæmatin is but oxidised hæmochromogen, a conclusion which is false, and which is an illustration of the mistakes into which observers may be led who conclude as to the identity of two colouring matters from the identity of prominent absorption-bands in their spectra.

A strong proof that oxidised hæmochromogen is not identical with hæmatin is derived from my own observations on the absorption of the extreme violet and ultra-violet. Whilst hæmatin possesses even in solutions of great dilution the power of absorbing the whole of the ultra-violet, the violet and even the blue

rays of the solar spectrum, oxidised hæmochromogen is, in solutions of much greater concentration, remarkably transparent for the ultra-violet.

Hoppe-Seyler made the observation that perfectly pure solutions of hæmatin are quite unaffected by reducing agents, but that the addition of certain foreign matters (*e.g.* albumin) renders reduction possible. I can, from my own repeated observations, emphatically confirm this fact.

It has been stated above that diluted blood and solutions of oxyhæmoglobin treated with acids exhibit a band in the red between C and D (of which the centre is approximately situated at λ 640), though it varies somewhat with the nature of the acid which has effected the decomposition. If, however, blood be treated with glacial acetic acid, and the mixture *at once* shaken with ether, the latter subsequently separates, holding so much of an acid compound of hæmatin in solution as to possess a deep red colour. This ethereal solution, in addition to the characteristic band of acid hæmatin, exhibits three other bands whose positions and relative intensities are indicated in Plate II., Spectrum 6.

Hæmatin hydrochloride (*syn.* **hæmin**).—When a minute drop of blood on a glass slide is mixed with a drop or two of glacial acetic acid, and the mixture is boiled over a tiny flame, and then allowed to evaporate, the residue is found on microscopic examination to contain innumerable reddish-brown prismatic crystals, which were formerly constantly referred to as Teichmann's[1] crystals (after their discoverer). Such crystals may be obtained from any old blood stain on cloth, linen, wood, metal, etc. The stained tissue or the scrapings of the stain are heated, as above, with glacial acetic acid. It is necessary, however, in the case of stains which may have been subjected to the action of water, to add a *minute* crystal of sodium chloride to the glacial acetic acid before boiling. Hoppe-Seyler[2] subsequently discovered methods of obtaining Teichmann's crystals in quantities, which enabled him to examine their physical properties with some degree of completeness and to analyse them, and he was able to show that hæmin is a compound of hæmatin and hydrochloric acid, to which, as a result of his more recent researches, he ascribed the empirical formula $C_{34}H_{35}N_4FeO_5HCl$. Nencki and Sieber,[3] on the other hand, assigned to hæmin the formula $C_{32}H_{30}N_4FeO_3HCl$, corresponding to the formula $C_{32}H_{30}N_4FeO_3$, which they assign to hæmatin.

Method of preparing hæmin in bulk.—A method for preparing hæmin in bulk was, as has been said, first devised by Hoppe-Seyler, and other methods were described by Nencki and Sieber. These methods demand the expenditure of much time, labour, and patience; and none of them, as I know from my own abundant personal experience, yield a product which can compare in the absolute uniformity of its crystallisation and the complete absence of all amorphous matter with the one described by Schalfijew, which is as follows:—

One volume of defibrinated and strained blood is added to four volumes of glacial acetic acid, previously heated to 80° C. As soon as the temperature has fallen to 55°–60°, the liquid is again heated to 80° C. On cooling, crystals *at once* separate, and can be seen floating in the liquid, presenting a charac-

[1] *Ztschr. f. rat. Med.*, 1853, Bd. iii. S. 375, and Bd. viii. S. 141.

[2] *Virchow's Archiv*, 1864, Bd. xxix. S. 597–600; "Das Hämin," *Med.-chem. Untersuch.*, Berlin, S. 379-385.

[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1884, Bd. xviii. S. 401; 1886, Bd. xxi. S. 325; 1888, Bd. xxiv. S. 430.

teristic silky lustre and a dark blue colour. The crystals are allowed to settle for at least twelve hours, and the clear dark brown mother-liquid is syphoned off. The blue sediment, if care was taken to avoid the presence of any blood clots in the defibrinated blood used in the preparation, is found on microscopic examination to be entirely composed of crystals of hæmin. It is repeatedly washed by decantation with water; then thrown on a filter, and, after renewed washing with distilled water, it is subjected to long-continued washing with spirit and ultimately with absolute alcohol. This washing with alcohol must be continued so long as the alcohol assumes a brown colour, and is a very long process. The blue mass remaining on the filter is ultimately washed with ether and alcohol, and in the first instance is allowed to dry by exposure to the air, and afterwards by heating to 115° C.[1]

It was stated by Schalfijew that by his process 5 grms. of pure hæmin can be obtained from 1 litre of defibrinated blood. This yield, which would be approximately equivalent to the theoretical yield, on the assumption that the blood contains in the mean 12 per cent. of hæmoglobin, is from my own experience never realised, 1 litre of blood yielding on the average 3·5 grms. of pure hæmin.

Physical and chemical properties.—Whilst presenting in mass a blue colour, and exhibiting, when floating in a liquid, a silky lustre, on microscopic examination hæmin crystals appear dark brown elongated rhombic plates and prisms belonging to the triclinic system. They are arranged singly or in groups. They are strongly doubly-refracting. They are quite insoluble in water, alcohol, ether, or chloroform.

When pure uniformly crystallised hæmin is boiled in pure glacial acetic acid, the latter dissolves an appreciable quantity, assuming a dark brownish-red colour. From this solution the hæmin is in great part deposited, on cooling, in perfect crystals, without any admixture with amorphous substances. I find, however, that if the process of re-crystallisation be repeated, the substance deposited on cooling consists of hæmin crystals mixed with some amorphous colouring matter.

Hæmin is very easily soluble in highly dilute solutions of the caustic alkalies and their carbonates; from these solutions hæmatin is precipitated on the addition of an acid. If nitric acid be used as the precipitant, the chlorine which had originally been combined with the hæmatin, and which is now present in the filtrate as an alkaline chloride, can be precipitated by silver nitrate.

When hæmin crystals are heated, they remain unchanged up to about 200° C.; more strongly heated, they glow and leave an ash composed of pure iron oxide. When pure hæmin is intimately mixed, as by pounding, with pure concentrated sulphuric acid, hydrochloric acid is liberated.

Nencki and Sieber, who employed amyl alcohol in the preparation of hæmin, found that when prepared in this way the crystals contained amyl alcohol, and that their composition corresponded to the formula $(C_{32}H_{30}N_4FeO_3.HCl)_4C_5H_9.OH$.

The existence of a definite compound of hæmin and amyl alcohol is, however, doubted by Hoppe-Seyler.[2]

[1] M. Schalfijew. I have not seen the original paper in the *Journ. russk. fiz.-chim. Obsh.*, St. Petersburg, 1885, S. 30–37. See abstracts in *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1885, Bd. xviii. (Referat Bd.), S. 232–233; also in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1885, Bd. xv. S. 138.

[2] "Ueber Blutfarbstoffe und ihre Zersetzungsproducte," *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. x. S. 331.

The compounds of hæmatin with acids, *e.g.* hæmatin-hydrochloride, present, even in solutions of great dilution (1 : 25,000–1 : 50,000), an intense absorption-band, which encroaches more and more on the ultra-violet, as the strength of the solution increases. In a solution containing one part of crystallised hæmatin hydrochloride in 20,000 parts of glacial acetic acid, the band extends between *h* and M, the most intense absorption between *h* and L. The less refrangible border of this band is sharply defined, whilst the more refrangible border is less definite. As the solution is diluted the band becomes narrower, through less and less of the ultra-violet being absorbed. In highly dilute solutions the band which is still intense absorbs both H and K.[1]

The acid compounds of hæmatin exhibit, therefore, an absorption-band, which is exactly on the boundary of the ultra-violet proper, and which extends further and further into the ultra-violet as the concentration of the solution increases.

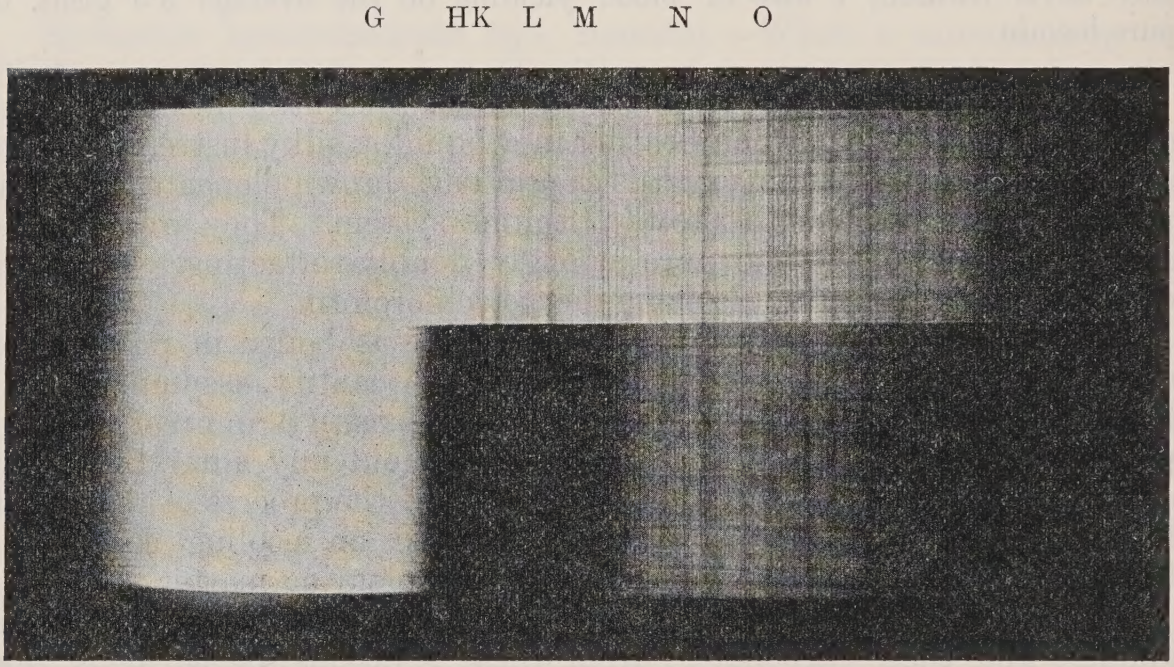

Fig. 37.—The photographic spectrum of hæmin.

Hæmochromogen (Syn. "Reduced Hæmatin").

It has already been explained that Hoppe-Seyler employed the name hæmochromogen to denote the very remarkable body which he was the first to study with care, and which results from the decomposition of reduced hæmoglobin, *in the absence of all oxygen*, by acids, and especially by alkalies, and of which the solutions present absorption-bands in the visible spectrum, which are identical with those of the reduced hæmatin of Stokes.

The latter name had been applied by Stokes to the chemical substance assumed to be the cause of the characteristic absorption-spectra which are exhibited by solutions of the blood-colouring matter, and likewise by impure solutions of hæmatin when subjected to the action of reducing agents. It now remains to describe the methods of preparing solutions of hæmochromogen, the body itself and its properties (so far as these are known to us), its combinations, and especially to refer to the views which Hoppe-Seyler advanced and held, in reference

[1] Gamgee, *Proc. Roy. Soc. London*, 1896, vol. lix. p. 276.

to the relations of hæmochromogen to hæmoglobin, and the part which it plays in relation to the optical properties of, and the chemical affinities for gases manifested by, the complex molecule of hæmoglobin.

Methods of preparing solutions containing hæmochromogen by the direct decomposition of hæmoglobin.—Without referring to a more complicated and in some respects more satisfactory method of decomposing hæmoglobin in the absence of oxygen,[1] the following very simple method, which, like the first, we owe to Hoppe-Seyler,[2] will be described.

A solution of oxyhæmoglobin is placed in a glass tube, and then a smaller glass tube containing a solution of sodium or potassium hydrate, or, if desired, of tartaric or phosphoric acid, is introduced into the larger tube, the open end of which is then drawn out and sealed in the blow-pipe flame. The apparatus thus prepared is then subjected to gentle heat, taking care not to incline the tubes so as to cause their contents to mix.

The oxyhæmoglobin contained in the larger, outer tube first becomes reduced, and thereafter the oxygen contained in the air of the tube is absorbed by the hæmoglobin. When many days have elapsed, and the whole of the hæmoglobin is again reduced, the tubes are inverted and their contents mixed, when the formation of hæmochromogen may be followed by the changes in colour and in the spectrum, which the colouring matter undergoes.

Physical and chemical properties.—When acted upon by dilute solutions of the caustic alkalies, hæmochromogen gives rise to a beautiful cherry-red solution, which, when sufficiently diluted, exhibits two absorption-bands apparently identical with those of Stokes' reduced hæmatin, which have already been referred to.

The visible spectrum of solutions of hæmochromogen in alkaline solutions is distinguished from all others by the extraordinary intensity and sharpness of the absorption-band nearest to D. The second absorption-band, which is very much less intense, has less sharply-defined borders. The solution, even when concentrated, absorbs very little of the red.

The following are measurements of the position of the absorption-bands in the visible spectrum by Hoppe-Seyler and myself:—

Gamgee's	measurements[3]	(1878)	λ 567–547	λ 532–518
Hoppe-Seyler's	„	[4] (1889)	λ 565–547	λ 527–514

My study of the photographic spectrum of hæmochromogen has led to the following results:[5]—Solutions, even of very great dilution, exhibit an absorption-band between *h* and *g*. This band has the same position as the band of CO-hæmoglobin, but is much more intense. With one part of hæmochromogen in 25,000 parts of water, a stratum 10 mm. thick being examined, an intense absorption-band occupies the region between λ410·0 and λ430·0. From the examination of solutions of various strengths it results that the mean ray absorbed corresponds to about λ 420·0.

By heating to 110° C. a solution of hæmochromogen mixed with a sufficiently concentrated solution of sodium hydrate, hæmochromogen

[1] Hoppe-Seyler, *Med.-chem. Untersuch.*, Berlin, S. 540 and 541; and Gamgee's "Physiological Chemistry," vol. i. pp. 118 and 119.
[2] "Physiol. Chem.," 1878, S. 390.
[3] "Physiological Chemistry," 1880, vol. i. p. 111.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 496.
[5] Gamgee, *Proc. Roy. Soc. London*, 1896, vol. lix. p. 276.

separates as a violet-grey powdery precipitate, which dissolves again in the liquid from which it had separated, as soon as this cools. It is quite erroneous to state, as is asserted in all text-books,[1] that Hoppe-Seyler succeeded in separating hæmochromogen in a crystalline condition. He only succeeded (*at most*) in obtaining crystals of the CO-compound, and *concluded* that hæmochromogen itself must be a crystalline body, but he never even asserted that he had actually obtained the crystals, and a promise made in 1889[2] to describe the assumed crystalline hæmochromogen, though implying that he had already obtained the body in this condition, was never fulfilled. Moreover, in the last systematic account of hæmochromogen which he published in 1893, Hoppe-Seyler[3] does not refer to its being crystalline, but, on the contrary, speaks of it (as he had done in 1889) as separating in the form of a violet-grey powdery precipitate.

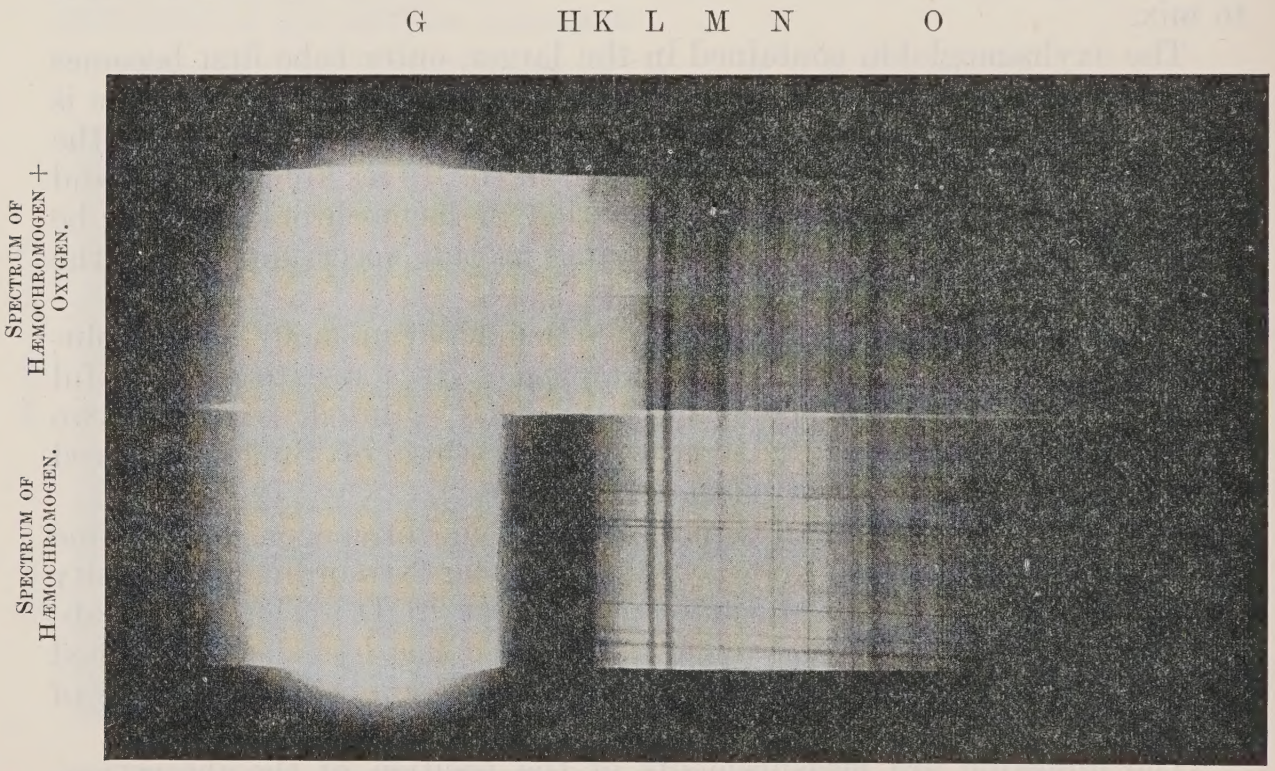

Fig. 38.—The photographic spectrum of oxygenized hæmochromogen and of hæmochromogen.

Acids, even when very dilute, lead in the first instance to the formation of hæmochromogen from reduced hæmoglobin, in the absence of oxygen; they, however, decompose a part of the hæmochromogen with great rapidity, removing its iron and giving rise to hæmatoporphyrin. This explains, according to Jäderholm,[4] the complex (four-banded) nature of the spectrum of hæmochromogen, as at first described by Hoppe-Seyler,[5] when prepared by the action of acids on hæmoglobin.

[1] Hammarsten, "Lehrbuch d. phys. Chem.," Dritte Auflage, 1895, S. 122; Neumeister, "Lehrbuch der physiol. Chem., etc.," 1895, Bd. ii. S. 154; Halliburton, "A Text-Book of Phys. Chemistry," 1891, p. 290; Sheridan Lea, "The Chemical Basis of the Animal Body," Appendix to Foster's "Physiology," 1892, p. 232.

[2] Hoppe-Seyler, *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 495.

[3] Hoppe-Seyler und Thierfelder, "Handbuch d. phys. u. path. Chem. Analyse," Berlin, 1893, S. 214, 215 ("Hämochromogen").

[4] See Abstract by Hammarsten in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, Bd. iv. S. 102.

[5] *Med.-chem. Untersuch.*, Berlin, S. 542. In his later descriptions of the spectrum of acid solutions of hæmochromogen no mention is made of four bands.

When subjected to the action of such reducing agents as tin and hydrochloric acid, hæmochromogen gives rise to coloured products, which are obviously nearly related to, though not identical with, such bodies as the so-called urobilins.

It was stated that when blood saturated with CO, or a concentrated solution of CO-hæmoglobin, is treated with a concentrated solution of sodium hydrate, a bright red precipitate separates. Jäderholm stated that this precipitate consisted of a compound of CO with hæmatin, and could be prepared directly by the action of the gas on a solution of reduced hæmatin; he further asserted that the visible absorption-spectrum of the CO-hæmatin closely resembled that of CO-hæmoglobin, the bands occupying the same position; though he described them as being less intense in the hæmatin compound, and as differing from the CO-hæmoglobin compound in the fact that the two bands α and β exhibit equal intensities.

By causing an alkaline hydrate to act upon CO-hæmoglobin in the absence of oxygen (method with double tubes previously described), and heating to 100° C., Hoppe-Seyler separated the body which Jäderholm had described as CO-hæmatin, but which appears really to be CO-hæmochromogen. Like hæmochromogen itself, its CO-compound, which has been deposited at 100° C., dissolves again when the liquid from which it separates cools. The CO-compound of hæmochromogen is described by Hoppe-Seyler as a crystalline body, though none of its physical characters have been subjected to even a superficial examination. The visible spectrum of its solution is, according to Hoppe-Seyler, absolutely undistinguishable from that of CO-hæmoglobin.

The most interesting and weighty observation made by Hoppe-Seyler on this subject was, however, that concerning the volume of CO which combines with hæmochromogen to form its CO-combination. He found that the same volume of CO combines with hæmochromogen as would be required to convert an equivalent weight of reduced hæmoglobin into the CO-compound. This unquestionably interesting observation, taken in connection with the fact that crystals form under certain circumstances in solutions which contain CO-hæmochromogen (there is no absolute proof that the crystals represent this substance), led Hoppe-Seyler to form certain hypotheses of extraordinary boldness, for which the experimental bases are as yet altogether wanting, but which have been accepted with misplaced confidence; these hypotheses he looked upon as legitimate conclusions from his own experiments, and formulated as follows:—

"We are justified in concluding that in crystallised CO-hæmoglobin, as well as in the colouring matter of the blood corpuscles, there is present a particular group of atoms which combines with and retains carbonic oxide, which is characterised by the special manner in which it absorbs light, and which, after separation from the albuminous residues, passes unchanged into CO-hæmochromogen.

"Without possibility of doubt, this group of atoms is identical with the one which, in the arterial blood-colouring matter,[1] and in crystallised oxyhæmoglobin, holds two atoms of oxygen in combination, in the place of a molecule of CO.

"The oxyhæmoglobins, the hæmoglobins, and the CO-hæmoglobins, as

[1] Reference is here made to the hypothetical "arterin."

well as the colouring matters of the red-blood corpuscles, all contain hæmochromogen, and this body can be obtained from them all by a process of simple decomposition, even in the crystalline condition, and almost in theoretical proportions."[1]

In other words, Hoppe-Seyler announced that, from his experiments it might be concluded that hæmochromogen represented an iron-containing coloured radical, which, by linking itself to an albuminous residue or albuminous residues, forms hæmoglobin, and that hæmochromogen in the latter body combining with a molecule of oxygen forms oxyhæmoglobin; with a molecule of carbonic oxide, carbonic-oxide hæmoglobin, etc.—these substances containing oxyhæmochromogen and CO-hæmochromogen respectively.

Not only are the facts wanting which would be needed in order to prove this hypothesis, but there are many others which appear to me to indicate that whilst, *when once formed*, hæmochromogen, as indeed hæmatin, includes the specific atomic group upon which the characteristic optical and physico-physiological properties of the blood-colouring matter depend, probably hæmochromogen does not exist preformed in hæmoglobin and its compounds. I trust shortly to throw more light on this question.

Linossier[2] described compounds of hæmatin and reduced hæmatin with nitric oxide as well as with carbonic oxide. On repeating his experiments, I convinced myself that (as had been shown by Jäderholm and by Hoppe-Seyler in the case of CO) NO exerts no action on hæmatin, but appears to form a compound with hæmochromogen, which is possessed, as Linossier describes, of a fine red colour, and exhibits two absorption-bands between D and E, similar to those of oxyhæmoglobin. This NO-hæmochromogen awaits a careful examination.

Hoppe-Seyler has speculated in reference to the condition in which the iron exists in hæmochromogen and hæmatin respectively, and has emitted the opinion that the iron in hæmochromogen is present in a ferrous and in hæmatin in a ferric condition, but the grounds for an opinion do not actually exist.[3]

Hæmatoporphyrin.

Methods of preparation. — When either hæmatin or hæmin is thoroughly mixed with concentrated sulphuric acid, it dissolves, and by filtering through asbestos a clear and beautiful purple-red solution is obtained. When this solution is poured into a large quantity of water, the greater part of the dissolved colouring matter is precipitated in the form of a brown flocculent precipitate, the quantity of which increases if alkalies be added so as to neutralise the acid. This colouring matter is impure hæmatoporphyrin. In this operation the acid separates the whole of the iron from the hæmatin, and it is found in solution in the state of a *ferrous* salt. In the process of decomposition of hæmatin by sulphuric acid there is no evolution of hydrogen gas.

From hæmatin and hæmin hæmatoporphyrin can also be obtained—(1) by the action of strong HCl in sealed tubes heated to 130° C.

[1] Hoppe-Seyler, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xiii. S. 492 and 493.

[2] "Sur une combinaison de l'hématine avec le bioxyde d'azote," *Compt. rend. Acad. d. sc.*, Paris, tome civ. p. 1296.

[3] For the discussion of the question, see Hoppe-Seyler, *Med.-chem. Untersuch.*, Berlin, S. 546–559.

(Hoppe-Seyler); (2) by the action of acetic acid saturated with HBr, aided by heat (Nencki and Sieber).

Hæmochromogen is, in the absence of oxygen, converted even by the weakest acids into hæmatoporphyrin, the iron being found in the solution as the ferrous salt of the acid employed. Although occurring more slowly, the decomposition of CO-hæmochromogen by acids also yields hæmatoporphyrin.

According to Hoppe-Seyler, the composition of hæmatoporphyrin is represented by the formula $C_{34}H_{38}N_4O_6$.

According to Nencki and Sieber, who have made the most complete investigation of this body, it has the composition $C_{16}H_{18}N_2O_3$, and they explain its origin from hæmatin by the following equation, in which they adopt their own as distinguished from Hoppe-Seyler's formula for hæmatin—

$$\underset{\text{(hæmatin)}}{C_{32}H_{30}N_4FeO_3} + 3H_2O = 2(C_{16}H_{18}N_2O_3 + Fe)$$

According to Nencki and Sieber, hæmatoporphyrin is isomeric with bilirubin.

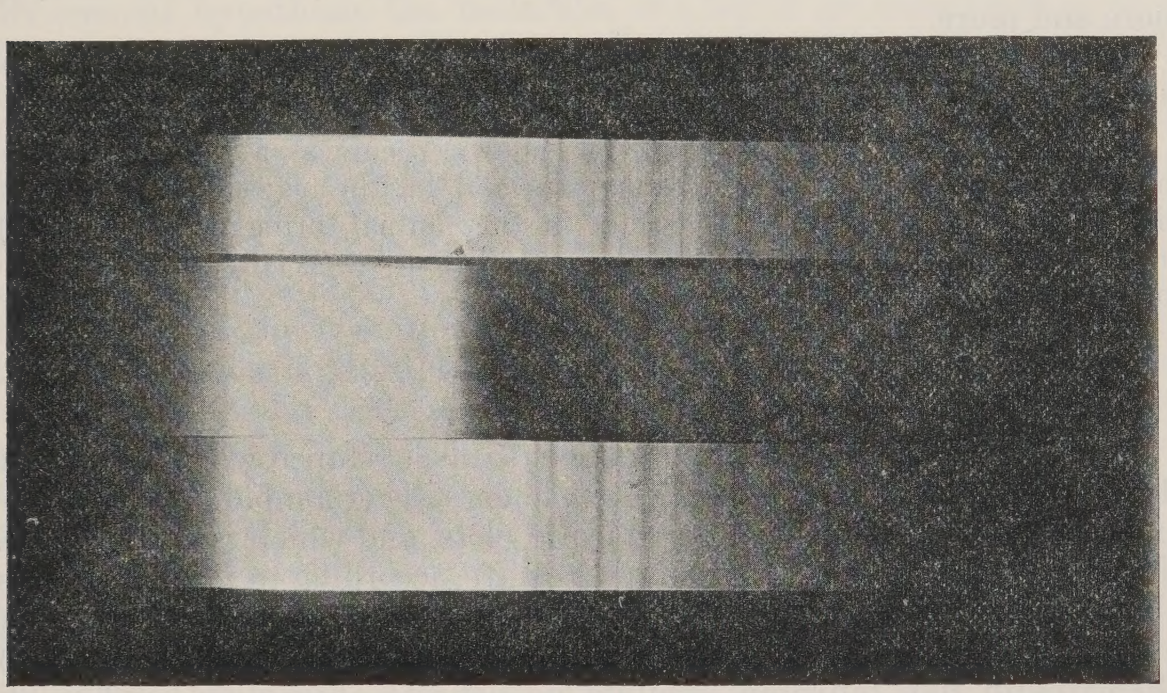

FIG. 39.—The photographic spectrum of hæmatoporphyrin.

Physical and chemical properties.—Hæmatoporphyrin forms beautiful crystalline compounds with Na and with HCl.

It is insoluble in pure distilled water, slightly soluble in dilute acids, more soluble in strong acids, and readily soluble in alkaline solutions, weak and strong. It is also readily soluble in acid and alkaline alcohol.

Solutions of hæmatoporphyrin in acidulated alcohol have a beautiful purple colour, and assume a bluish violet tint when the solution is made very strongly acid. Alkaline solutions are of a fine red, but in the presence of a great excess of alkali exhibit a violet tint. Solutions of hæmatoporphyrin, even if extraordinarily dilute, exhibit a magnificent red fluorescence, which strangely enough is not referred to in text-books, though it seems to me to be their most remarkable characteristic.

An alcoholic solution of hæmatoporphyrin, acidulated with hydro-

chloric or sulphuric acids, exhibits in the visible spectrum two absorption-bands, of which one, which is the narrower and the weaker, is situated between C and D and immediately adjoins D. The second, which is much more intense, more sharply defined and broader, lies nearly midway between D and E; but nearer the former than the latter.

Alkaline solutions exhibit in the visible spectrum four absorption-bands, to wit, a weak band midway between C and D, an equally weak band between D and E, but nearer to the former, a more strongly marked band nearer to E, and lastly a fourth band, darkest of all, which occupies four-fifths of the interval between B and F.

The spectra of acid and alkaline hæmatoporphyrin are exhibited in Fig. 57.

A study of the photographic spectrum of hæmatoporphyrin has given me the following results:[1]—Acid solutions of hæmatoporphyrin, so dilute as to appear colourless (though presenting, if examined in a dark room by means of a beam of sunlight reflected from the mirror of the heliostat, the marked red fluorescence previously referred to), exhibit an intense absorption-band between *h* and H. If the solution be slightly more concentrated, K is absorbed, and with increasing concentration of the solution the absorption of the ultra-violet extends more and more.

Alkaline solutions of hæmatoporphyrin absorb the same spectral region, but the intensity of the absorption is greater.

Hæmatoporphyrin, as MacMunn has shown, occurs as a colouring matter in the integument of some invertebrates and in the egg-shells of certain birds.[2] In small quantities it occurs in the normal urine (Arch. Garrod), and in larger quantities in certain toxic conditions, especially in one of the forms of chronic sulphonal poisoning.

Hæmatoidin.

This name was applied by Virchow to a substance which occurs in the form of orange-coloured microscopic crystals (rhombic plates) in old extravasations of blood, as in apoplectic clots, and which is certainly derived from hæmoglobin. These crystals are, according to most observers, identical in form with those of bilirubin, and when treated with fuming nitric acid exhibit the same colour reaction (Gmelin's reaction). Hæmatoidin, like bilirubin, exhibits no definite absorption-band in its spectrum, but effects a general absorption of the ultra-violet, violet, and blue rays of the spectrum. Opinions were long divided on the question of the identity or non-identity of hæmatoidin and bilirubin, but they are now generally regarded as identical.

Certain other substances (of which the chemical history is very imperfect), which can be directly obtained by the action of reagents on the blood-colouring matter, and certain pigments occurring in the organism, and which, on grounds more or less satisfactory, have been held to be derived from it likewise, will be considered in the account of the chemistry of the urine as well as in that of the chemical processes occurring within the alimentary canal.

[1] *Proc. Roy. Soc. London*, 1896, vol. lix. p. 279.

[2] MacMunn, *Journ. Physiol.*, Cambridge and London, 1885, vol. vii. p. 240; vol. viii. p. 384.

A GENERAL ACCOUNT OF THE PROCESSES OF DIFFUSION, OSMOSIS, AND FILTRATION.

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Diffusion.

By current hypothesis the molecules of a liquid are considered to be in constant motion, so that if two liquids, miscible without chemical interaction, are placed in contact, a mutual interpenetration, without the action of any external force, takes place; or, in other words, a diffusion of the molecules of one among those of the other, and *vice versâ*, occurs, the process tending to continue until in the final state a homogeneous mixture of the two exists. In physiological problems we deal with the diffusion of substances in dilute aqueous solution, and it must at once be noted that the condition of the molecules of a substance in dilute aqueous solution is probably different in the case of different substances, and by no means necessarily the same as that of the undissolved substance; that, in fact, the solvent and dissolved substance in many cases interact, with a resultant alteration of physico-chemical properties.

In the case of substances acting as electrolytes in aqueous solution, it is believed that dissociation into the ions takes place to a greater or less extent of the total number of molecules, according to the degree of dilution.[1] There will thus be at lower degrees of dilution a mixture of molecules, *active* as regards electrolytic conduction and chemical action, and *inactive* molecules, the latter tending to become active by ionic dissociation as dilution is increased, so that at infinite dilution only active molecules exist in the solution. *The coefficient of activity* will be the number expressing the ratio of active molecules to the total of active plus inactive, and is unity at infinite dilution. The electrical conductivity of a solution of an electrolyte is dependent on the velocity of migration of its ions,[2] so that the ratio of the *molecular conductivity*[3] of a solution of an electrolyte at given dilution, to the limiting value

[1] Arrhenius, *Bihang. till k. Svens. Vet.-Akad.*, Stockholm, 1884, Bd. viii., Nos. 13 and 14; *Ztschr. f. physikal. Chem.*, Leipzig, 1887, Bd. i. S. 631.

[2] Kohlrausch, *Ann. d. Phys. u. Chem.*, Leipzig, 1879, Bd. vi. S. 1, 145; 1885, Bd. xxvi. S. 161.

[3] The molecular conductivity is the ratio of the conductivity to the molecular concentration of the solution, the latter being the ratio of grammes per litre to the molecular weight in grammes.

which this approaches on increasing dilution, is a measure of the coefficient of activity of the solution. According to this view, then, a very dilute solution of sodium chloride consists of positively-charged sodium and negatively-charged chlorine ions moving amongst the water molecules, but unable to part company by virtue of their charges of opposite sign, and only separable by the application of energy from without (electrolysis). Other substances which do not conduct electricity in aqueous solution are believed to be in a simpler state of solution, the molecules moving among those of the solvent not being known to be in a different condition to those of the undissolved substance, but simply capable of freer motion.

It is further probable that in the case of certain non-electrolytes in solution, instead of single molecules we deal with aggregates of molecules, and such substances are said to be in colloidal solution (κόλλα, glue). As instances of organic substances the aqueous solutions of which are colloidal, may be mentioned albumin, gum-arabic, starch, hæmoglobin.[1]

It must at once appear likely that the ease with which the "molecules" of different substances can move among those of the solvent in a solution is different in the case of different substances, *i.e.* that the power of *diffusibility* must be very variable.

Graham[2] gives the following table:—

Equal weights had diffused to the same extent in the following times:—

Hydrochloric acid .	1	Magnesium sulphate .	7
Sodium chloride .	2·33	Albumin . . .	49
Cane-sugar . .	7	Caramel . . .	98

Substances in solution tend to diffuse from places of higher to those of lower concentration, and in the law of Fick[3] it is stated that the quantity of dissolved substance so diffusing is proportional to the rate of fall in concentration.

Thus, if a is the quantity of substance passing section q of a diffusion cylinder in time z, when at x the concentration in the section is c, and at $x+dx$ is $c+dc$; then—

$$a = -kqz\frac{dc}{dx}$$

where k is a constant peculiar to the substance and known as the *coefficient of diffusion.*

From the law of Fick, Stefan[4] calculated for a special case the following formula:—

$$a = cq\sqrt{\frac{kz}{\pi}}$$

[1] Picton and Linder (*Journ. Chem. Soc.*, London, 1892, vol. lxi. p. 148; 1895, vol. lxvii. p. 63) have prepared solutions of arsenious sulphide of various "grades." Thus one may have (α) aggregates visible by microscope; (β) no visible aggregates, but the substance not diffusible; (γ) the substance diffusible but not filterable; (δ) the substance both diffusible and filterable, but the aggregates still large enough to scatter light. They consider that in matter in solution one can pass by grades from obvious suspension, to colloidal solution, to non-electrolytic crystallised solution, and so to the first grade of electrolytic solution.

[2] *Phil. Trans.*, London, 1861, vol. cli. p. 183.

[3] *Ann. d. Phys. u. Chem.*, Leipzig, 1855, Bd. xciv. S. 59.

[4] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1879, Bd. lxxix. S. 161.

where a is the amount of substance, passing in time z, through section q, from an infinitely long cylinder of solution of concentration c, into another such cylinder of pure solvent.

This formula was experimentally verified by Voigtländer [1] with cylinders of agar jelly, in which diffusion occurs as easily as in water.[2] He further investigated the temperature coefficient (α) for k, and found that it is not a linear function of the temperature, as stated by Weber,[3] but stands in the following relation [4]:—

$$K_t = K_o\,(1 + \alpha t)^2$$

In the case of an electrolyte in solution, the diffusion must be considered as that of the ions into which it is dissociated on passing into solution. The velocity of the separated ions may be very different, but since in solution by virtue of their opposite charges they cannot part, the more rapidly moving ion must be retarded by the more slowly moving, and the more slowly moving accelerated by its more active fellow. The diffusion of an electrolyte may also be accelerated by the presence in the liquid into which it is diffusing, of ions charged oppositely to those forming the more active partner in the diffusing substance. Thus hydrochloric acid diffuses faster into a solution of sodium chloride than into water.[5] As a rule, those electrolytes which are the best conductors, are the most diffusible in solution.[6] The presence of a substance that is not an electrolyte in the fluid into which diffusion is taking place may slow the diffusion of an electrolyte. Thus sodium chloride diffuses more slowly into sugar solution than into water, and the presence of ethyl alcohol also retards its diffusion.[7] In the case of non-electrolytes in solution, diffusion must concern the "molecules" of the dissolved substance, and the "aggregates" of colloids will find their way with greater difficulty than the "molecules" of crystalloids.

No definite rule can be stated as regards the effect of concentration of the solution upon the rapidity of diffusion of the dissolved substance. With sodium chloride the coefficient of diffusion is practically unaltered by change in concentration of the solution. In the case of magnesium sulphate the coefficient falls with the concentration of solution, while with hydrochloric, nitric, and sulphuric acids the coefficient rises with the concentration.[8]

The simultaneous diffusion of two salts, studied first by Graham, has been since more completely investigated by Marignac.[9] In general the rapidity of diffusion of the more diffusible of a pair of salts diffusing simultaneously is found to be increased, that of the less diffusible diminished.

In the following table the diffusions of five pairs of salts, separately and simultaneously, are contrasted.

[1] *Ztschr. f. physikal. Chem.*, Leipzig, 1889, Bd. iii. S. 316.
[2] Graham, *Ann. d. Chem.*, Leipzig, 1862, Bd. cxxi. S. 5, 29.
[3] *Ann. d. Phys. u. Chem.*, Leipzig, 1879, Bd. vii. S. 536.
[4] For the values of α, which vary slightly with different substances, see Voigtländer's original paper, *loc. cit.*
[5] Arrhenius, *Ztschr. f. physikal. Chem.*, Leipzig, 1892, Bd. x. S. 51.
[6] Long, *Ann. d. Phys. u. Chem.*, Leipzig, 1880, Bd. ix. S. 613; Lenz, *Mém. Acad. imp. d. sc. de St. Pétersbourg*, 1882, tome vii. p. 30.
[7] Arrhenius, *loc. cit.*
[8] Scheffer, *Ztschr. f. physikal. Chem.*, Leipzig, 1888, Bd. ii. S. 390.
[9] *Ann. de chim.*, Paris, 1874, Sér. 5, tome ii. p. 546.

r is the ratio of the diffusion coefficients of the two salts, with separate diffusions.

r' is the ratio with simultaneous diffusions.

R, the ratio of the amounts diffused of the same salt in separate and in simultaneous diffusion, *i.e.* the alteration of the coefficient of diffusion produced by the presence of the other salt.

	Separate.	Simultaneous.	r.	r'.	$r' : r$.	R.
NaCl	·5833	·6054	1	1	...	1·038
Na_2SO_4	·3770	·2497	·590	·352	·596	·662
KCl	·8560	·9276	1	1	...	1·083
$BaCl_2$	·8433	·4424	·572	·401	·701	·814
NaCl	·7142	·7883	1	1	...	1·019
$BaCl_2$	·5673	·5225	·757	·668	·882	·921
K_2SO_4	·4745	·4378	1	1	...	·901
$MgSO_4$	·2028	·1684	·382	·345	·903	·830
Na_2SO_4	·3757	·3420	1	1	...	·910
$MgSO_4$	·2097	·1823	·523	·502	·960	·869

As a rule, as seen in R, the more diffusible salt is accelerated, the less diffusible delayed. In the two last pairs both members are delayed, but the less diffusible more markedly.

In the body it is rare to find the conditions present for a free diffusion between the constituents of two solutions; a membrane, whether composed of cells or the surface layer of the protoplasm of a cell, as a rule intervenes, and obviously the permeability of the membrane affects the result. If pig's bladder separates methyl alcohol and ether, the methyl alcohol diffuses into the ether, but if a caoutchouc membrane separates the two liquids, the ether diffuses into the alcohol.[1]

Osmosis.

The term *osmosis* is applied to diffusion taking place between two liquids separated by a membrane.

The simplest case of this is that in which a solution of a substance is separated from the pure solvent by a membrane permeable by the solvent but impermeable by the dissolved substance. Such membranes were first prepared by Traube,[2] in the form of colloidal precipitates, such as tannate of gelatin and ferrocyanide of copper, but Pfeffer[3] was the first to thoroughly study the process of osmosis under such conditions. The name "semipermeable" has been given to such membranes, but it must be noted at once that this expression is seldom strictly accurate and must always be used relatively to some particular substance. Tamman[4] has pointed out that such membranes are by no means the "molecule sieves" that Traube imagined,[5] and in experimental work the membrane must be chosen to suit the substance, or *vice versâ*. Copper ferrocyanide forms one of the best of such membranes, and is nearly impermeable to cane sugar.

[1] Raoult, *Ztschr. f. physikal. Chem.*, Leipzig, 1885, Bd. xvii. S. 735.
[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1867, S. 87 and 129.
[3] "Osmotische Untersuch.," Leipzig, 1877.
[4] *Ztschr. f. physikal. Chem.*, Leipzig, 1892, Bd. x. S. 255.
[5] See also Walden, *ibid.*, 1892, Bd. x. S. 699.

In practice such membranes are formed in the interstices of an indifferent supporting structure, such as the pores of a porous battery pot (preferably previously soaked in gelatin), by placing one of the membranogens inside the pot, which is then lowered into a solution of the other, so that the precipitate is formed within the structure of the earthenware where the two solutions come into contact. It is only by such an artifice that the membrane can be sufficiently supported to enable it to withstand the high pressure produced by the osmosis under the conditions.[1]

If, now, a battery pot with such a membrane in its pores be filled with a solution of sugar in water, hermetically sealed, and placed in a vessel of water, the water molecules will diffuse in either direction through the membrane, which is permeable to them; the sugar molecules, on the other hand, cannot pass out, for to them the membrane is impermeable. As a result of the presence of the sugar on the inner side of the membrane, in unit time, more water enters the pot than passes out, and the pressure rises until it is sufficient to bring about the condition of equality in the number of water molecules entering and leaving the pot.

This pressure is called *the osmotic pressure* of the solution of sugar, under the conditions of concentration and temperature. That this pressure is comparable to that of a gas was first clearly pointed out by van 't Hoff.[2]

Thus the osmotic pressure of a dilute solution at constant temperature is proportional to its concentration (*i.e.* density of a gas in the law of Boyle). This is illustrated by the following table from Pfeffer:[3]—

Cane Sugar Solutions at 13°·5 *C. to* 16°·1 *C.*

Concentration of Solution.	Osmotic Pressure in Mm. of Hg.	$\frac{\text{Osmotic Pressure}}{\text{Concentration.}}$
1 per cent.	535	535
2 ,,	1016	508
2·74 ,,	1518	554
4 ,,	2082	521
6 ,,	3075	513

Again, at constant concentration of a dilute solution, the osmotic pressure is proportional to the absolute temperature (law of Charles).

Thus, again, taking Pfeffer's data—

1 *per Cent. Cane Sugar Solution.*

	Temperature.	Observed Pressure.	Calculated Pressure.
		Mm. Hg.	Mm. Hg.
(1)	32°	544	...
	14°·15	510	512
(2)	36°	567	...
	15°·5	520·5	529

[1] For details of manufacture see Adie, *Journ. Chem. Soc.*, London, 1891, vol. lix. p. 344.

[2] *Arch. neérl. d. sc. exactes, etc.*, 1885, Bd. xx. S. 239; *Ztschr. f. physikal. Chem.* Leipzig, 1887, Bd. i. S. 479.

[3] *Loc. cit.*, p. 85.

The experiments of Soret,[1] again, show that in a solution, as in a gas, the warmest part is the most dilute. Soret introduced a solution into a long vertical tube and maintained a difference of temperature at the two ends, the upper end being warmer than the lower. At the end of several weeks the concentration of the solution at the warm end of the tube was found to be lowered. Thus, with solution of copper sulphate, the concentration at the end of the tube at 20° C. was 17·332 per cent., while that at the end maintained at 80° C. was 14·03 per cent., instead of 14·3 per cent. as calculated by Charles' law. And, again, with concentration of 29·867 per cent. at the 20° C. end, a concentration of 23·871 per cent. was found at the end warmed to 80° C. instead of 24·8 per cent. as calculated.

Thus "the osmotic pressure of a dissolved substance is exactly the same as the gas pressure, measured by the manometer, which one would observe if he could remove the solvent, and leave the dissolved substance as a gas filling the same volume."[2] The hypothesis of Avogadro then is, according to van 't Hoff, not merely capable of extension by the law of Henry to solutions of gases, but to solutions of matter which is not gaseous under ordinary circumstances, and it may be stated that equal volumes of gases or dilute solutions at the same gas or osmotic pressure, and at the same temperature, contain equal numbers of molecules.

A marked concordance is seen in the table below, between the observed osmotic pressures for sugar solution taken from Pfeffer[3] and those calculated on the hypothesis of Avogadro and the law of Charles.

One per cent. sugar solution contains 1 grm. of sugar in 100·6 c.c. of solution. At the same temperature and pressure, $\frac{2}{342}$ of a grm. of hydrogen contains by hypothesis the same number of molecules ($C_{12}H_{22}O_{11} = 342$).

Taking the weight of a litre of hydrogen, at 0° C. and one atmosphere pressure, as ·08956 grm., and the above concentration as ·0581 grm. per litre, the gas pressure at 0° C., at the volume 100·6 c.c., is ·649 atmosphere, and at the temperature t = ·649 (1 + ·00367t).

Temperature.	Observed Osmotic Pressures.[4]	Calculated Gas Pressures ·649 (1+·00367t).
6°·8 C.	·664	·665
13°·7 C.	·691	·681
14°·2 C.	·671	·682
15°·5 C.	·684	·686
22° C.	·721	·701
32° C.	·716	·725
36° C.	·746	·735

The law of Dalton may also be applied, with certain restrictions, to the osmotic pressure of solutions, the total pressure of a mixture of substances being equal to the sum of the partial osmotic pressures of the several components.

[1] *Arch. d. sc. phys. et nat.*, Genève, Sér. 3, tome ii. p. 48 ; *Ann. de chim.*, Paris, Sér. 5, tome xxii. p. 293.

[2] Nernst's "Theoretical Chemistry," 1895, Palmer's trans., p. 148.

[3] *Loc. cit.*

[4] Pfeffer, *loc. cit.*, p. 85.

The following instances are taken from Pfeffer:[1]—

Copper Ferrocyanide Membrane.

Concentration.	Rise of Fluid in Measuring Tube in Mm. Per Hour.	
	Experiment I. Temp. 17°·1 C.	Experiment II. Temp. 15°·8 C.
	Mm.	Mm.
1 per cent. saltpetre . . .	6·08	5·4
15 ,, gum-arabic . .	2·06	1·8
1 ,, saltpetre + 15 per cent. gum-arabic	7·90	7·0
1 per cent. saltpetre . . .	6·06	5·3

Parchment Paper Membrane.

Concentration.	Rise of Fluid in Measuring Tube in Mm. Per Hour.	
	Experiment I.	Experiment II.
1·5 per cent. calcium chloride .	9·9	10·3
2 ,, gum-arabic . .	1·2	1·3
1·5 ,, calcium chloride + 2 per cent. gum-arabic . .	11·4	11·3

Temperature in both experiments, 17°·4 C.

In cases, however, where the two constituents of the solution have a common ion, each salt diminishes the dissociation of the other, so that the pressure of the mixture is less than the sum of the pressures of the two components.[2]

Thus for a double salt—

	A.		*B.*	
	Osmotic Pressure.	Sum of Components.	Osmotic Pressure.	Sum of Components.
$\frac{1}{40}(NH_4)_2SO_4$ . .	1·264 At.	...	1·295	...
$\frac{1}{40}Al_2(SO_4)_3$ . . .	1·265 ,,	...	1·22	...
$\frac{1}{40}K_2SO_4$ . . .	1·29 ,,	...	1·40	...
$\frac{1}{40}(NH_4)_2Al_2(SO_4)_4 24Aq$	2·37 ,,	2·53	1·98	2·515
$\frac{1}{40}K_2Al_2(SO_4)_4 24Aq$.	2·39 ,,	2·56	1·96	2·62

[1] Pfeffer, *loc. cit.*, p. 68.
[2] From Adie, *Journ. Chem. Soc.*, London, 1891, vol. lix. p. 344.

It is, however, by no means the fact that, in the case of all substances in aqueous solution, agreement exists between the observed osmotic pressure and that directly calculated on the above hypothesis alone. In many cases the pressures observed in solution are far higher than those calculated from the concentration in gramme-molecules per unit volume. Thus the osmotic pressure of a 1 per cent. aqueous solution of common salt at 0° C., by calculation on the above data, should be 3·79 atmospheres, but actual measurement shows it to be over 7 atmospheres.

This phenomenon, common to all solutions of electrolytes, is accounted for on the hypothesis of Arrhenius,[1] that the dissociated ions of an electrolyte in solution are capable of exerting pressure as well as the undissociated molecules. The osmotic pressure of solutions of electrolytes is then raised above the simple molecular value by the coefficient expressing the extent to which the molecules are dissociated in passing into solution (*dissociation coefficient*).

This coefficient gives the ratio of the observed osmotic pressure of a solution to the pressure calculated on the assumption that no dissociation of molecules occurs in passing into solution. It may be determined for a substance at a particular dilution most accurately, by measurement of the electrical conductivity of the solution.

If m is the number of *inactive* molecules in the solution, and n the number of *active*, and k the number of ions into which a molecule can be dissociated, then the dissociation coefficient $i = \frac{m + k.n}{m + n}$

Since the "activity co-efficient" $\alpha = \frac{n}{m+n}$ is measurable by the ratio of the molecular conductivity of the solution to the limiting value it approaches by increased dilution, $i = 1 + (k - 1)\alpha$ can be obtained by measurement of conductivity of solution. i can obviously also be obtained from measurements of osmotic pressure.

This coefficient will necessarily be of very different value for different classes of electrolytes, since the possible number of ions is variable. Thus sodium chloride has 2, potassium sulphate 3, potassium ferrocyanide 5 ions.

Hence as a formula may be given—

$$P = 22{\cdot}35\ (1 + {\cdot}00367t)\ \frac{c}{m}\ i \text{ atmospheres},$$

where 22·35 atmospheres is the pressure exerted by the gramme-molecule of gas in volume of 1 litre at 0° C., c the number of grammes of the substance per litre, m its molecular weight, and i its dissociation coefficient at the concentration c.

As regards the practical estimation of the osmotic pressure of a solution, the direct measurement by a semipermeable membrane is not only tedious, and limited to cases where the dissolved substance has no chemical action on the film, but seldom practicable, on account of the difficulty in constructing membranes, to which the term may be strictly applied. Obviously, unless the membrane is really impermeable to the dissolved substance, the values on account of the "leakage" of dissolved substance must be below the real amount.

[1] *Ztschr. f. physikal. Chem.*, Leipzig, 1887, Bd. i. S. 631.

Blagden [1] discovered the fact that the freezing-point of a solution is lower than that of the solvent, and that the lowering of freezing-point is proportional to the concentration of the solution. Rüdorff,[2] Coppet,[3] and Raoult [4] have since more thoroughly investigated the matter. If, therefore, we know the lowering of the freezing-point of water, produced by the addition of a gramme-molecule to the litre (1°·89 C.), and the osmotic (or gas) pressure at 0° C. corresponding to this (22·35 atmospheres), it is merely a matter of simple proportion to calculate the pressure at 0° C. corresponding to any given lowering of freezing-point, and from that to obtain the pressure at any other temperature by the law of Charles.

Many pieces of apparatus have been devised for measuring the lowering of the freezing-point, but that of Beckmann [5] is in most general use. Unfortunately, the method does not yield concordant results in the hands of different observers (when aqueous solutions are used) within about 005° C., which corresponds to an osmotic pressure of about 50 mm. of mercury at the temperature of the body (37° C.), and is hence of little value for the correct estimation of small differences of osmotic pressure in the aqueous solutions to which the physiologist confines his attention.[6]

An optical method has been used by Tamman.[7] If a drop of solution of potassium ferrocyanide is allowed to fall into a solution of copper sulphate, a so-called "Traube cell" is formed, the ferrocyanide solution within which is separated from the copper sulphate solution outside by a precipitation membrane of copper ferrocyanide, through which osmotic interchange can take place.

If the internal solution be of higher osmotic pressure than the external, water passes from the copper solution outside into the cell, and the copper solution immediately round about the cell, being raised in concentration, tends to sink. In the reverse case, by dilution of the layer round the cell, an upward current is started. There are thus produced differences in the refractive index of the layer of solution against the outside of the cell, in contrast to the rest of the copper solution. These are easily detected by the Töpler *Schlierenapparat.*[8] If the ferrocyanide solution have the same osmotic pressure as the copper solution, no *schlieren* will be produced, and there will be no change in refraction. Now, since the total osmotic pressure is the sum of the partial pressures, a third substance, not reacting with the membranogens, may be added to the solution of one of them, and the concentration of the other, isosmotic with the mixture, determined by the method. Since the osmotic pressure of the solution of the membranogens, to which the third substance was added, is directly measurable, it is obvious that the partial pressure of the added substance can be measured.

[1] *Phil. Trans.*, London, 1788, vol. lxxviii. p. 277.

[2] *Ann. d. Phys. u. Chem.*, Leipzig, 1861, Bd. cxiv. S. 63 ; 1862, Bd. cxvi. S. 55 ; 1871, Bd. cxlv. S. 599.

[3] *Ann. de chim.*, Paris, 1871, Sér. 4, tome xxiii. p. 366 ; 1872, tome, xxv. p. 502 ; 1872, tome xxvi. p. 98.

[4] *Ibid.*, Paris, 1884, Sér. 6, tome ii. p. 66 ; *Compt. rend. Acad. d. sc.*, Paris, 1882, tome xcv. p. 1030.

[5] *Ztschr. f. physikal. Chem.*, Leipzig, 1888, Bd. ii. S. 638.

[6] Loomis, *Ann. d. Phys. u. Chem.*, Leipzig, 1894, Bd. li. S. 500 ; Jones, *Ztschr. f. physikal. Chem.*, Leipzig, 1893, Bd. xi. S. 110 ; Raoult, *ibid.*, 1892, Bd. ix. S. 343.

[7] *Ztschr. f. physikal. Chem.*, Leipzig, 1888, Bd. ii. S. 415.

[8] *Ann. d. Phys. u. Chem.*, Leipzig, 1867, Bd. cxxxi. S. 33.

Physiological methods of estimating osmotic pressure have also been devised. The method of de Vries[1] is based upon the plasmolysis of the protoplasts of vegetable cells. The cells filled with coloured sap from the middle nervure of the leaf of *Tradescantia discolor* are useful for the purpose, sections of this part being allowed to soak for three to five hours in the solutions whose osmotic pressures are to be determined. If the cells are plasmolysed, *i.e.* if the protoplasts are found on examination to have shrunk from the cell walls, the osmotic pressure of the solution producing this effect is above that of the cell sap, for water has passed from the latter to the former, as evidenced by the diminution in volume. By investigating a series of solutions with sections from the same leaf, it is of course possible to find two of slightly differing concentration of the substance under investigation, one of which just causes plasmolysis, while the other (weaker) does not. A solution of concentration equal to the mean of these two is said to be *isotonic* with the cell sap.

De Vries, on preparing a number of solutions of different substances, all isotonic with the same batch of cells, and expressing their concentrations in gramme-molecules to the litre, found that it required a lower gramme-molecular concentration of some substances than of others to obtain isotony. The term "water extracting power" (*Wasseranziehungsvermögen*) was used to express this peculiarity which is obviously related to what has above been termed dissociation. Taking 0·1 grm. molecule to the litre of saltpetre as a standard, and giving it a magnitude of 3, the relative value (as regards plasmolysis of vegetable cells) of a molecule of a number of substances was expressed in terms of that of a molecule of saltpetre, and the numbers expressing this ratio called *isotonic coefficients* of the substances.

Thus, to barium chloride ($BaCl_2 + 2Aq = 244$) is given the isotonic coefficient 4, which means that $\frac{3}{4}$·244 parts by weight of barium chloride in aqueous solution exert the same plasmolysing action as 101($KNO_3 = 101$) parts by weight of saltpetre *i.e.* a 1·83 per cent. solution of crystallised barium chloride is isotonic by the method, with a 1·01 per cent. solution of potassium nitrate.

Since cane sugar on this system is given the value of 2 for its isotonic coefficient, and since, being a non-electrolyte, it is not dissociated in solution, it is merely necessary to divide the isotonic coefficients of de Vries by 2, in order to obtain ordinary dissociation coefficients.

It is obvious that the substances in solution must exert no deleterious action on the protoplast of the cell, and must, moreover, be quite unable to diffuse through it, if the method is to be exact.

Here, again, we are met with the difficulty, that the protoplast is not a strictly semipermeable membrane. It must let certain substances pass, otherwise the cell sap could not have any other constituent than water; and it is only because the permeability to certain substances is so far below that to water, that it is possible to obtain fairly approximate measures of osmotic pressure by this method. With other substances the permeability is so great that the values are far too low.

Thus with sodium chloride, by this method, the dissociation coefficient is reckoned as 1·5 (de Vries' isotonic coefficient 3), but by lowering of

[1] *Jahrb f. wiss. Botanik*, 1884, Bd. xiv. S. 427; *Ztschr. f. physikal. Chem.*, Leipzig, 1888, Bd. ii. S. 415.

freezing-point method, it is found to be higher (about 1·89). Other indices of "isotony" than the plasmolysis of the vegetable cell have been used by physiologists.

Hamburger[1] has used red blood corpuscles. If these are placed in solutions of substances which do not penetrate, and which do not act chemically upon them, an index of the entrance of water into the substance of the corpuscle is presented by the setting free of hæmoglobin, which is recognisable in the solution. In a solution of lower osmotic pressure than the corpuscle-contents (hypoisotonic solution), water enters the corpuscles and the solution is reddened. In a solution of higher osmotic pressure than the contents (hyperisotonic solution), water is extracted from the corpuscles, they shrivel and sink, and the solution retains its original colour. Two limiting solutions are thus obtainable, and the mean concentration of the two is taken as that isosmotic with the contents of the corpuscle.

The method has very considerable limits in practice, for not only is it obviously restricted to colourless solutions, but it can also only give results approaching the truth, in cases where the substance in solution does not penetrate; and, as indicated by Gryns,[2] who has criticised the method very severely, the red corpuscles are penetrable by a very large number of substances.[3]

Another blood corpuscle method is that of the hæmatokrit.[4] Here the gauge of entrance or exit of water from the corpuscles is the volume they occupy, in a graduated capillary tube, after having been centrifugalised with the solution. The volume of the corpuscles is dependent on the osmotic pressure of the solution in which they are placed (provided the dissolved substance does not penetrate), and if equal volumes of the same blood specimen, contemporaneously centrifugalised in two solutions of different substances, give the same volume of corpuscles, those solutions have the same osmotic pressure. By centrifugalising a given volume of a blood sample in a series of solutions of a substance not penetrating corpuscles (cane sugar), of different and known osmotic pressure, in separate tubes, at the same time as an equal volume of the same blood treated with the solution of the substance to be investigated, a final comparison of the length of the "threads" of corpuscles in the tubes gives a gauge of the osmotic pressure of the solution.

By centrifugalising blood in a pipette, previously oiled (cedar oil) to prevent clotting, measuring the length of the "thread," and comparing with the same, blood treated with sugar solutions of known osmotic pressure, the pressure of the plasma is determinable and is found to vary, rising after meals, and especially after the ingestion of solutions of salt (Koeppe).

A bacterial method even has been used by Wladimiroff,[5] who has

[1] *Arch. f. Anat. u. Physiol.*, Leipzig, 1886, Phys. Abth., S. 476; 1887, S. 31; *Ztschr. f. physikal. Chem.*, Leipzig, 1890, Bd. vi. S. 319.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 86.

[3] Hamburger himself (*Ztschr. f. physikal. Chem.*, Leipzig, 1890, Bd. vi. S. 319) maintained that permeability did not affect his method, since, by a "vital act" ("Lebenserscheinung"), S. 331, the corpuscles give up to the solution from their juice an amount of some other substance exactly equivalent to that which penetrates from without, so that the total osmotic pressure of the juice is unaltered!

[4] Hedin, *Skandin. Arch. f. Physiol.*, Leipzig, 1890; Gaertner, *Berl. klin. Wchnschr.*, 1892, Bd. xxix. S. 36; Koeppe, *Arch. f. Physiol.*, Leipzig, 1895, S. 154; *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxii. S. 567; *München. med. Wchnschr.*, 1893, No. 24.

[5] *Ztschr. f. physikal. Chem.*, Leipzig, 1891, Bd. vii. S. 529.

maintained that cessation of motion of bacteria placed in solutions indicates that the solution is isosmotic with the cell sap, in cases where poisonous action can be excluded.

The above physiological methods of measuring osmotic pressure are of considerable interest, but, as already stated, their application is decidedly limited; and though, as will appear below, often of indirect value, as giving information bearing on the permeability of cells and membranes, they are not to be classed with the more accurate methods of experimental physics.

In the case of colloid solutions, it is not necessary to use precipitation membranes for the direct measurement of osmotic pressure, for such material as vegetable parchment is, as a rule, impermeable to colloids, and it moreover presents certain advantages in particular cases, namely, those in which the colloidal substance is contaminated by salts (*e.g.* albuminous solutions), since the salts can pass out, and the determination is freer from the error of inclusion of the partial pressure of these, unavoidable by a direct measurement by a copper ferrocyanide membrane, or an indirect determination by lowering of freezing-point.

It is known that solutions of colloids of considerable concentration exert very low osmotic pressure, though their exact measurement is difficult. Picton and Linder,[1] in a direct measurement (by a copper ferrocyanide membrane) of the pressure of a 4 per cent. solution of colloidal arsenious sulphide, obtained a pressure of only 17 mm. of water. Sabanejew[2] states that the lowering of freezing-point by silicic acid is so small as to be within the limits of the method. With albuminous solutions the difficulty of contaminating salts is almost insuperable, and since the molecular weight of albumin is not known, calculation is excluded.

Sabanejew[3] investigated the lowering of freezing-point of water by solution of egg albumin, and quotes a lowering of ·02° C. for a 15·6 per cent. solution, and ·042° C. for a 30·35 per cent. solution, but since the specimens held ·4 to ·66 per cent. of ash, the numbers are of no value. Tamman[4] gives the difference in lowering of freezing-point of horses' serum, produced by coagulation of the proteids by heat and removing them, as only ·006° C., which is in the region of the error of the method.[5] Dreser[6] and Koeppe[7] also state that the removal of proteid from albuminous solutions does not affect the osmotic pressure, while Lüdeking[8] maintains that the boiling point of 40 per cent. solution of gelatin is 100° C.[9] It is therefore uncertain whether proteids in

[1] *Journ. Chem. Soc.*, London, 1895, vol. lxvii. p. 63.

[2] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1890, Bd. xxiii. S. 87.

[3] *Ibid.*, 1891, Bd. xxiv. S. 558.

[4] *Ztschr. f. physikal. Chem.*, Leipzig, 1896, Bd. xx. S. 180.

[5] Starling, on the other hand, quotes two experiments to prove that the osmotic pressure of the proteids of serum can be directly measured. It is stated to be from 30 to 40 mm. of Hg. *Journ. Physiol.*, Cambridge and London, 1895, vol. xix. p. 323. Cf. also next article, p. 308.

[6] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1892, Bd. xxix. S. 314.

[7] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxii. S. 571 (footnote).

[8] *Ann. d. Phys. u. Chem.*, Leipzig, 1888, Bd. xxxv. S. 552.

[9] A lowering of vapour pressure (raising of boiling point) is produced by solution of a substance in a solvent, and the lowering of vapour pressure, like that of the freezing-point, is proportional to the concentration. Wüllner, *Ann. d. Phys. u. Chem.*, Leipzig, 1858, Bd. ciii. S. 529; 1858, Bd. cv. S. 85; 1860, Bd. cx. S. 564; Tamman, *Ann. d. Phys. u. Chem.*, Leipzig, 1888, Bd. xxxiv. S. 299.

colloidal solution exert an osmotic pressure capable of measurement by our present methods.

From the above account of osmotic pressure, it is evident that, since it is present in high or low degree in all true solutions, as a result of the kinetic energy of the dissolved molecules, the phenomena of diffusion are most satisfactorily accounted for as directly dependent on the osmotic pressure exerted by the diffusing substance.[1] Substances diffuse from places of higher to those of lower partial pressure, and the differences in rapidity of diffusion of different substances, though present in concentrations exerting the same osmotic pressure, must be accounted for by differences in the resistance met in their passage among the molecules of the solvent.

When we now turn to the consideration of the interchange of the constituents of solutions through animal membranes, we at once find that, since these membranes are never strictly semipermeable, and are frequently very permeable for dissolved substances, the phenomena are neither those of pure osmose nor pure diffusion, but a complex of the two, in which the relative permeability of the membrane to solvent and dissolved substance is of paramount importance, but, unfortunately, a variable factor with different membranes.[2] All the earlier work upon osmosis was carried out with membranes not fulfilling the condition of semipermeability, so that a double stream of solvent into solution (endosmose) and dissolved substance into solvent (exosmose) was considered as a necessary feature of the process until Traube's discovery of precipitation membranes.

The first osmose experiment was probably that of the Abbé Nollet,[3] in which it was observed that a bladder tied over a vessel of spirits of wine became distended, or even burst, when vessel and membrane were under water. Parrot[4] again called attention to the fact, which had been forgotten, and ascribed the process to "affinity of the first order," which causes all miscible fluids to "wander" into one another. Fischer[5] in Germany and Dutrochet[6] in France again rediscovered the prime fact, and commenced its systematic study. Certainly the main stimulus to subsequent study of the phenomena was given by the work of Dutrochet.[7] Dutrochet's endosmometer was a funnel closed by membrane and provided with a long stem. The body of the funnel was filled with the solution, and the whole immersed in water. The height to which the fluid rose in the stem was the gauge of the osmotic action of the solution. Dutrochet recognised that the concentration of the solution and the temperature affected the results.

Vierordt[8] improved upon the arrangement used by Dutrochet, by setting the membrane vertical and the stem horizontal, so that filtration error was avoided, and also concluded that the stream of water into the

[1] Nernst, *Ztschr. f. physikal. Chem.*, Leipzig, 1888, Bd. ii. S. 611.

[2] In this connection see a paper by Lazarus Barlow, *Journ. Physiol.*, Cambridge and London, 1895, vol. xix. p. 140.

[3] "Histoire de l'Académie royale des sciences," 1748, p. 101.

[4] *Ann. d. Phys. u. Chem.*, Leipzig, 1815, Bd. li. S. 318.

[5] *Ibid.*, 1822, Bd. lxxii. S. 300.

[6] *Ann. de chim.*, Paris, 1827, tome xxxv. p. 393; "Agent immédiat du mouvement vital," Paris, 1826.

[7] See also "Mémoires pour servir à l'histoire anatomique et physiologique des végétaux et des animaux," Bruxelles, 1837.

[8] *Ann. d. Phys. u. Chem.*, Leipzig, 1848, Bd. lxxiii. S. 519.

funnel was proportional to the difference of concentration of the solutions on either side of the membrane.

Jolly[1] specially studied the ratio between the amount of water passing into the solution and the amount of dissolved substance passing out, using salts with pig's bladder as membrane. This ratio he termed the *endosmotic equivalent* of the salt, and maintained that it is constant for the same membrane, concentration of the salt solution, and temperature. For some years after this the whole attention of those interested in the matter of osmosis was directed to a fuller study of this ratio in the case of different substances.[2]

As a result of these researches, it was seen that even with the same membrane it was only within slight changes of concentration of the solution that constancy of the endosmotic equivalent was obtainable, a result in accordance with expectation, seeing that the physical nature of an animal membrane must necessarily undergo change with the amount of water imbibed, a quantity variable with the concentration of the solutions in which it is in contact. With a strictly semipermeable membrane, the endosmotic equivalent is evidently infinite, while the more permeable the membrane to dissolved substance the lower will be the equivalent. Thus, according to Harzer,[3] the endosmotic equivalent for sodium chloride is with fish-swim-bladder, 2·9 ; ox-pericardium, 4·0 ; ox-bladder, 6·4.

It must therefore be admitted that, in spite of the great labour that has been expended on the determination of endosmotic equivalents of different substances with different membranes, the results obtained are of little value to the practical physiologist, who deals with membranes in the living body, whose physical characters are by no means necessarily those of the structures used in such experiments. The only value that can be attached to these determinations is an orienting one, as to the diffusibility of the substances into water, through dead animal membranes, under the conditions of the experiments.

Before we can attempt to answer the question, How is the process of diffusion modified when in an osmose experiment an animal membrane is placed between solution and solvent? it is obviously necessary to know the physical structure of the membrane. Of this we must admit great ignorance. To Brücke[4] we owe a theory of "pore diffusion." Assuming capillary pores in the membrane, it maintains that, by attraction, a layer of pure water lines these, while an axis of salt solution, whose concentration falls from axis to mantle of the cylindrical pore, lies centrally. The highest concentration in the axis must be that of the salt solution in the experiment, and along the axis ordinary hydrodiffusion takes place, water entering the salt solution and salt entering the water. Along the mantle, however, only water can pass into the salt solution, so that the stream of water exceeds that of salt. If the pores are very narrow, it is conceivable that there is no central core of salt solution, in fact the membrane becomes semipermeable.

[1] *Ztschr. f. rat. Med.*, 1849, Bd. vii. p. 83 ; *Ann. d. Phys. u. Chem.*, Leipzig, 1849, Bd. lxxviii. S. 261.

[2] Fick, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1857, Bd. iii. S. 294 ; W. Schmidt, *Ann. d. Phys. u. Chem.*, Leipzig, 1857, Bd. cii. S. 122 ; *Beitr. z. Anat. u. Physiol. (Eckhard)*, Giessen, 1855, Bd. i. S. 97 ; 1860, Bd. ii. S. 1, 31, 147 ; Hoffmann, *ibid.*, 1860, Bd. ii. S. 59.

[3] *Arch. f. physiol. Heilk.*, Stuttgart, 1856, Bd. xv. S. 194.

[4] "De diffusione humorum per septa mortua et viva," Berlin, 1842 ; *Ann. d. Phys. u. Chem.*, Leipzig, 1843, Bd. lviii. S. 77.

The attraction of the substance of the membrane for water, at any rate, may then be a factor in the case. Ludwig[1] demonstrated, indeed, that the concentration of the solution imbibed by an animal membrane may be lower than that of the solution in which it is soaked.

Fick[2] distinguished between two possibilities for diffusion through an animal membrane — a "pore diffusion" in Brücke's sense, and a diffusion occurring through the spaces between the molecular aggregates of which the membrane may be considered to be built. The latter idea is somewhat of the nature of that formed of the diffusion of a gas through a film of liquid in which it is soluble, or is perhaps better illustrated in the experiment of L'Hermite,[3] in which, when water separates chloroform from ether in a tube, the chloroform increases at the expense of the ether. Fick's "homogeneous" membranes were made of collodion; but his results show that such a membrane is not unalterable, since the amount of salt passing through increases with time, and it is difficult to escape the conclusion that in many cases some interaction of chemical nature takes place between the membrane and the substances to which it is permeable.[4]

The property possessed by certain substances of imbibing certain liquids (apart from capillary action), must be borne in mind in all considerations of the essential nature of the processes involved in the passage of fluids through membranes. This property can only be ascribed to some "affinity" between the molecules of the imbibing substance and that imbibed; thus gelatin swells in water but not in ether, while the reverse is true of caoutchouc. The retention of a gas, or a colouring matter by charcoal, of water by the silica of the opal, or that of pepsin by fibrin, are instances of the class of phenomena to which attention is here called, and to which the name of *adsorption* is often applied. When a homogeneous substance imbibes a solution, compounds of the imbibed with the imbibing substance may be formed, which may have a greater affinity for the solvent than the original imbibing substance, but at the same time the osmotic pressure of the solution tends to retard the imbibition of the solvent; hence, with a given pair of substances, the amount of the solution of one taken up by the other will reach a maximum at a certain concentration, a maximum, however, which may be well above that for imbibition of the pure solvent.

The "affinity" of the imbibing substance for the solvent and dissolved substance imbibed may be of very different order, for gelatin takes up a more concentrated solution of methyl-violet than that in the dye-bath; while, on the other hand, a ferrocyanide of copper membrane will take up water while almost absolutely indifferent to dissolved cane sugar.

Such "affinities" are not purely mechanical, since they vary with the chemical nature of the substances, and yet are not of the nature of chemical affinity in the usual sense of the term, since the "compounds" do not obey the laws of constant and multiple proportion. Ostwald has introduced the term *mechanical affinity* to meet the case.

In the complex known as protoplasm there may be imbibing substances of different nature, permeated by a solution of substances whose chemical nature may, directly and indirectly, affect the imbibition of a solution brought in contact with the mass; and, furthermore, undissolved particles may themselves

[1] *Ztschr. f. rat. Med.*, 1849, Bd. viii. S. 1; *Ann. d. Phys. u. Chem.*, Leipzig, Bd. lxxviii. S. 307.

[2] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1857, Bd. iii. S. 294.

[3] *Ann. de chim.*, Paris, 1854, Sér. 3, tome xliii. p. 420.

[4] Tamman, *Ztschr. f. physikal. Chem.*, Leipzig, 1892, Bd. x. S. 255; Walden, *ibid.*, S. 699.

exert surface action, so that the possibilities for purely physical absorption are quite unknown, and so-called *vital* elective action may be the result of specific adsorptive affinity. Hofmeister [1] has shown that gelatin has an "elective" action, for common salt, the concentration of the solution imbibed exceeding that of the surrounding solution; and, further, that the combination of sodic chloride with the gelatin favours the uptake of water. Again, gelatin takes up more water from ·5 to 2 per cent. solution of ethyl-alcohol in water than from pure water.

With salts that undergo electrolytic dissociation in solution, permeability must be a function of ions. Thus, according to Ostwald,[2] copper ferrocyanide is permeable to potassium chloride, because both chlorine and potassium ions can pass; it is impermeable to barium chloride, because the barium ion is stopped; and impermeable to potassium sulphate, because the sulphuric acid ion cannot pass; and, under ordinary circumstances, on account of opposite electrical charges, if one ion is stopped, so must be the other. There are, however, conditions under which an ion, stopped on account of the impermeability of the membrane to its fellow in a salt, may pass the membrane.

If the negative ion of a salt is prevented from passing through the membrane, only because it is impermeable to its positive fellow, the addition of another salt, whose positive ion can pass the membrane, will allow the negative ion of the first salt to pass in company with it. Or a salt whose negative ion can pass the membrane may be placed on the opposite side, the two negatives exchanging with their positive fellows across the membrane, and equal numbers of the two negative ions passing in opposite directions in a given time. This is of interest to the physiologist, since it opens a possible physical explanation of the fact that a cell may hold back a substance under certain conditions, while under others, when surrounded by a differently constituted fluid, the same substance may be given up.

Koeppe [3] has attempted to apply this to the formation of hydrochloric acid in the stomach from sodium chloride, maintaining that the stomach wall is impermeable to chlorine ions, but that the sodium ions are exchanged for hydrogen ions from the blood. That free hydrogen ions are present in the alkaline blood is, however, hardly possible.

Whether permeability be a function of physical or chemical nature, it is obvious that in the case of a living membrane the complex to which the term "physiological condition" is applied must affect the property, so that one and the same membrane in the body may, under different circumstances, be more or less permeable by the same substance.

The simplest living membrane with which experiments can be made is probably the differentiated outer layer of the protoplast of the vegetable cell (*Plasmahaut*). There is no doubt that the permeability of this membrane for different chemical substances is very variable. It is penetrated by some dye-stuffs but not by others, very impermeable to many simple salts, though easily permeable by certain complex organic substances.[4] Since this membrane is in its living condition so slightly permeable to salts, the osmotic pressure within vegetable cells is high (3 to 4 atmospheres). This special relative impermeability to salts is obviously regulated in some manner by the "physiological condition" of the membrane. Jansen [5] found that the cell sap of the alga, *Chæto-*

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 210.

[2] *Ztschr. f. physikal. Chem.*, Leipzig, 1890, Bd. vi. S. 71.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxii. S. 567.

[4] Pfeffer, *Abhandl. d. math.-phys. Cl. d. k. sächs. Gesellsch. d. Wissensch.*, 1890, Bd. xvi. S. 149.

[5] *Verhandl. d. k. Akad. v. Wetensch.*, Amsterdam, 1888, vol. iv. p. 345.

morpha, growing in sea water, is practically isosmotic with that of *Spirogyra*, growing in fresh water, though the osmotic pressure of sea water is some 240 times that of fresh. Thus the osmotic pressure of the cell sap of *Chætomorpha* is far below that of the water in which it lives, while that of the sap of *Spirogyra* is far above that of fresh water.

One can investigate the permeability of the living protoplast by the plasmolytic method already alluded to above. The cell is plasmolysed with a solution of some substance indifferent to the protoplasm and known not to penetrate (sugar). A solution of the substance to be tested is now prepared of the same osmotic pressure as the solution of indifferent substance which just causes plasmolysis. If the solution so prepared has exactly the same effect as the standard, it cannot pass through the protoplast, for, if it did, there would no longer be equality of osmotic pressure on the two sides thereof. If the substance to be tested is only slightly soluble in water, or is poisonous to the protoplasm, a small amount of it is added to the standard indifferent solution, and the effect of the addition on the plasmolysis noted. If it does not pass the membrane, then, by virtue of the higher osmotic pressure due to its addition, the mixture will produce more plasmolysis than did the standard solution, and the effect will be lasting. If no effect results from the addition, it must pass quickly through the membrane; if a passing effect, with subsequent recovery, it must pass slowly.

In this way Overton[1] has investigated the permeability of the protoplast by a number of chemical substances, and finds that salts much dissociated in solution hardly pass the membrane, while many complex organic bodies rapidly penetrate, and that the presence of certain radicles in these markedly affects the result.

In animal cells investigations are rather limited (by the fact that there is no plasmolysis) to shrinkage and swelling and escape of hæmoglobin (in red corpuscles), as indices of permeability, under conditions of variation of osmotic pressure of surrounding solutions. More, therefore, is known about the permeability of the red corpuscle than any other cell. A table of substances is given by Gryns,[2] and we here confine ourselves to stating that red corpuscles are permeable to urea,[3] glycerin, ethyl- and methyl-alcohol, and most ammonium salts (not to sulphate, phosphate, and thiocyanate), impermeable to sugars, sodium and potassium salts, barium and calcium chlorides, glycin and asparagin.

Thus, as regards action on red blood corpuscles, dilution of an isosmotic sodic chloride solution with urea solution produces the same effect as dilution with water, because the urea diffuses at once into the interior of the corpuscle, while, on the other hand, addition of sugar at once causes contraction of the cell.

Obviously, therefore, a so-called "hyperisotonic" solution does not necessarily extract water from a cell, and absorption of water from such a solution by the blood may be a purely physical action, if the substance in solution can permeate the wall separating it from the blood. Again, a drug, by making the wall of a cell less permeable by virtue of its chemical action on the protoplasm, may markedly affect the "water extracting power" of a salt solution. Possibly the fact that some salts

[1] *Ztschr. f. physikal. Chem.*, Leipzig, 1897, Bd. xxii. S. 189.
[2] *Loc. cit.*, p. 102.
[3] See also Schöndorff, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 192.

in the intestine purge (sodic sulphate), while others do not (sodic chloride), may in the end find its explanation in a permeability of the membrane by the latter, but not by the former. A universal "physiological salt solution," then, if by such a term is meant a salt solution in which tissues neither lose nor take up water, and the dissolved substance of which does not enter the cells, is not a possibility; each tissue must in fact have its own "normal solution,"[1] and this may possibly be in some cases a solution of some other substance than a salt.

The effective osmotic pressure, therefore, exerted against membranes such as those in the body which are, as a rule, partially permeable to dissolved substances, is far below that measured by a semipermeable membrane, and freezing-point determinations of osmotic pressures (determinations which give a gauge of the full osmotic pressure as it would be exerted against a semipermeable membrane), are of but orienting value to the physiologist, except in cases where the permeability of the membrane to the substance in solution is known.

The following table from Pfeffer[2] is illustrative of the diminution in the estimate of the full osmotic pressure caused by substituting a permeable membrane (bladder or parchment paper) for copper ferrocyanide, and it is evident that the effect is far more marked in the case of the crystalloids (saltpetre and sugar) than in that of the colloid (gum).

Six per cent. Solution of	Parchment Paper.	Bladder.	Copper Ferrocyanide.
Gum arabic . .	17·9	13·2	25·9
Cane sugar . .	29·0	14·5	287·7
Saltpetre . .	20·4	8·9	700·0 (not directly estimated.)

The pressures are in cms. of mercury.

The conditions, then, for the interchange of water and the constituents of solutions through membranes in the body, are evidently exceedingly complex, and it is at present practically impossible to assess the value of all the factors. Broadly stated, the following factors are concerned:—

1. The quantitative composition of the solutions separated by the membrane, and consequently the partial osmotic pressure exerted by the several constituents.
2. The coefficients of diffusion of the various constituents.
3. The permeability of the membrane in its physiological condition to the constituents.
4. The circumstances affecting the relative concentrations of a constituent on the two sides of the membrane with time, *e.g.* circulation and stirring.
5. The hydrostatic pressure on the two sides of the membrane.
6. The temperature.

The partial osmotic pressure of the constituents of a solution is obtainable from a quantitative analysis, if the molecular weight and

[1] Koeppe, *Arch. f. d. ges. Physiol.*, Bonn, 1897, Bd. lxv. S. 492.
[2] "Osmotische Untersuch.," Leipzig, 1877, S. 73.

dissociation coefficient (in case of electrolytes) is known. If the molecular weight is not known, as in the case of proteids, the substance must be removed from the solution, and the difference in the total osmotic pressure so produced estimated.

The coefficients of diffusion must be obtained under the special conditions (*e.g.* diffusion into serum, etc.).

The permeability of the membrane to dissolved substances, one of the most important factors, and one generally not capable of accurate estimation, will not only affect the passage of water and dissolved substances across the membrane by osmotic action, but also the hydrostatic pressure necessary to cause filtration.

We shall here content ourselves with considering a simple but usual case of absorption of a solution by blood, namely, one in which the osmotic pressure of the solution is lower than that of the blood, and the membrane separating the two permeable to the substance in solution, and to one at least of the constituents of the blood, but impermeable to others. For convenience the dissolved substance is called x, and that constituent of the blood to which the membrane is permeable, y. The blood, by virtue of its superior osmotic pressure, tends to take up water from the solution, and at the same time x diffuses through the membrane into the blood, and y into the solution. If the blood be first supposed to be stationary, a time is arrived at when the partial pressure of x and y is the same on either side of the membrane; in other words, this solution of x and y is now the "solvent" in an osmotic experiment, and the substances in the blood to which the membrane is impermeable are the "dissolved substances." The whole of x, of y, and the water of the original solution, must therefore in the end be absorbed.[1] If the blood, however, is circulated, the conditions for absorption are at once improved, for the diffusion of x into the blood is favoured by the fact that its partial pressure in the blood is kept low by renewed supplies of blood, by the stirring action of the corpuscles preventing the formation of "wall layers," and by the fact that cells in other parts of the body are enabled to take up the substance as it is brought round. It is also evident from the above that if, as a rare case, the solution had a higher osmotic pressure than the blood, provided only the membrane separating the two is permeable to the dissolved substance, and impermeable to some constituents of the blood, when once the solution has taken up enough water from the blood, and lost enough of its dissolved substance to the blood, to lower its osmotic pressure to that of the blood, the process described above is gone through, and it is in the end all absorbed.

For such absorption to be carried out completely, it is evident that the osmotic pressure of those constituents of the blood to which the membrane and capillary wall are not permeable, must exceed the pressure necessary to cause filtration across the same structures, for if the available osmotic pressure on the inner side of the capillary wall is less than the difference between the hydrostatic pressure on the two sides of the membrane, filtration must occur, and the solution can never be totally absorbed.

The assumption is here made that the resistance to the passage of fluid across the membrane is the same in both directions. It must be

[1] For this explanation to hold good, the substances in the blood to which the membrane is impermeable must be in true solution, and capable therefore of exerting osmotic pressure.

noted, however, that animal membranes are known in which the resistance to the passage of fluid is quite different in opposite directions.

The most familiar example is the shell membrane of the egg, which permits filtration far more easily from within outwards than in the reverse direction,[1] and the same is true of the skin of the frog.[2]

Filtration.

By filtration is meant the passage of fluid through a membrane, as a result of a difference of hydrostatic pressure on the two sides. To filter water from a solution across a membrane into another solution, the difference between the hydrostatic pressures on the two sides must exceed the difference between the osmotic pressures of the solutions, in the case where the higher osmotic and hydrostatic pressures are on the same side. If a porous pot bearing a semipermeable membrane is filled with a solution and immersed in the pure solvent, the pressure necessary to produce filtration of the solvent from the solution is one just exceeding the full osmotic pressure of the solution; but where we deal with permeable membranes, as those in the body, the necessary pressure is far less, because the difference of osmotic pressure on the two sides of the membrane is reduced by the diffusion of some of the dissolved substance.

Experiments on filtration through animal membranes appear to have given very contradictory results, which seems to be due to the fact that not only does continued pressure upon such membranes vary their permeability, but a certain amount of "recovery" takes place in the intervals between use; hence the conditions of the membranes have been by no means uniform in the experiments of different observers.

If the passage of fluid through an animal membrane is by more or less tortuous paths, and if the walls of these are more or less elastic, it is obvious, not only that by continued pressure must the resistance to filtration rise, but that on removal of the pressure a slow "recovery" will take place. Deformation of the membrane, if simply tied over a tube, must also, unless it is properly supported, tend to distort channels and so increase resistance to filtration. To these sources of difference in the experiments must be added, previous drying of the membrane or not, the condition of imbibition of the fibrous tissue, and temperature variations. All these sources of differences are, moreover, accentuated by the fact that most of the experiments have been made with thick membranes, such as pericardium, bladder, intestine, and ureter.

Nearly all who have studied filtration have found that at constant pressure the amount of the filtrate falls off with time,[3] but, as pointed out by Tigerstedt and Santesson,[4] this falling off is far more rapid in the earlier hours of an experiment than later, so that it is advisable in all such experiments on filtration to expose the membrane to pressure for

[1] Meckel quoted by Ranke, "Physiologie des Menschen," 1872, S. 122.

[2] Cima, *Mem. d. Accad. di Torino*, 1853, vol. xiii.; Reid, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 312.

[3] Liebig, "Untersuch. ü. einige Ursachen der Säftebewegung im thierischen Organismus," Braunschweig, 1848, S. 7; *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1858, Bd. i. S. 95; Runeberg, *Arch. d. Heilk.*, Leipzig, 1876, Bd. xviii. S. 58; Gottwalt, *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 423; v. Regéczy, *Arch. f. d. ges. Physiol.*, Bonn, 1883 Bd. xxx. S. 544.

[4] *Bijhang. till k. Svens. Vet.-Akad.*, Stockholm, 1886, Bd. xi., No. 2.

many hours before the observations are conducted, and the stage of relatively constant rapidity of filtration (a uniform rate is never actually attained) is reached quicker at higher than at lower pressure. This phenomenon is not due to any stopping of pores by particles suspended in the fluids, since it is not noted when filtration is effected through unglazed porcelain, and is probably simply a result of compression of tortuous channels.

The quantity of filtrate rises with the pressure, but in lower ratio.

Thus, in an experiment by Tigerstedt and Santesson[1] of filtration of distilled water through goldbeater's skin (serosa of ox-gut), which had previously been exposed for 95 hours to a pressure of 80 cms. of water,

Pressure.	Filtrate per Minute Observed.	Filtrate per Minute if proportional to Pressure.
20 cm.	·046 grm.	·046 grm.
40 ,,	·081 ,,	·092 ,,
60 ,,	·110 ,,	·138 ,,
80 ,,	·148 ,,	·184 ,,

The experiments of Wilibald Schmidt[2] showed a contrary result, *i.e.* that the filtration rapidity increased at a higher ratio than the pressure (possibly due to using dried membranes, the pores of which were opened during experiment), as also did those of v. Regéczy.[3]

A period of rest, interpolated between two filtration experiments at the same pressure, is found to often cause an increase of the permeability of the membrane above the value it possessed at the time of discontinuing the first experiment (Eckhard, Runeberg, Tigerstedt and Santesson).

Thus Tigerstedt and Santesson,[4] in a filtration of distilled water through gold-beater's skin at 40 cm. pressure, observed a filtrate of ·490 grm. per minute, but after a resting period of 530′ the filtrate at the same pressure was ·577 grm. per minute. But whether or not this phenomenon is observed, is probably due to whether or not the elastic limits of the fibres have been passed, "recovery" not being possible if the membrane has been excessively stretched. The interpolation of a period of filtration at lower pressure of course produces the same effect.[5]

The rapidity of filtration rises with the temperature,[6] and, according to Schmidt, the temperature coefficient is nearly that of Poiseuille, for the flow of fluids in capillary tubes.

The nature of the solution to be filtered must obviously affect both the rapidity of filtration, from differences in viscosity, and also the quantitative composition of the filtrate in relation to that of the original solution.

The following experiment from Tigerstedt and Santesson[7] may be quoted in evidence of the first point:—

[1] *Loc. cit.*, p. 31.
[2] *Ann. d. Phys. u. Chem.*, Leipzig, 1856, Bd. xcix. S. 337; 1861, Bd. cxiv. S. 337.
[3] *Loc. cit.* [4] *Loc. cit.*, p. 30. [5] Runeberg, *loc. cit.*
[6] Schmidt, *loc. cit.*; Eckhard, *loc. cit.*; Löwy, *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 537.
[7] *Loc. cit.*, p. 42.

1. *Egg albumin*—4·14 per cent. proteids; pressure, 32·5 cm.; goldbeater's skin.
(Filtrate per minute in grms., ·820, ·051, ·008, ·003, ·002, ·002.)

2. *Ox serum*—4·5 per cent. proteids; pressure, 30·5 to 34 cm.; goldbeater's skin.
(Filtrate per minute in grms., 2·586, 1·594, 1·000, ·812.)

In neither case had the membrane been previously stretched.

According to Gottwalt,[1] the serum albumin of blood serum filters through a ureter more easily than the globulin. Martin[2] has shown that homogeneous membranes of gelatin and gelatinous silicic acid form filters impermeable to solutions of many colloids, but as permeable to certain crystalloids as to water.

The relation of the concentration of the filtrate to that of the original solution is perhaps the most important point to the physiologist in the matter of filtration through animal membranes. There is general agreement that in the filtration of crystalloids the concentration of the filtrate is very nearly that of the original solution, and this appears to obtain at very various filtration pressures.[3] There is also general agreement that in filtration of colloids the concentration of the filtrate is always less than that of the original solution.[4] But as regards the effect of pressure on the concentration of a colloid filtrate, the results of different observers are not in accordance. Runeberg[5] has maintained that the concentration of the filtrate is higher at lower than at higher pressures, and the following table, taken from his later paper,[6] is illustrative :—

Fresh Sheep's Intestine—Ascitic Fluid (circulated) holding 3·72 per cent. of Proteids.

Time.	Pressure in Cm. of Fluid.	Filtrate per hour in Grms.	Per Cent. Albumin in Filtrate.
8 P.M. to 8.15 A.M.	90	1·84	2·34
8.15 A.M. to 2.15 P.M.		1·85	1·86
2.15 P.M. to 8.15 P.M.		1·97	1·60
8.50 P.M. to 8.50 A.M.	30	1·29	2·02
8.50 A.M. to 3.0 P.M.		1·52	2·12
4.0 P.M. to 7.30 P.M.	90	7·30	1·44
7.30 P.M. to 9.30 A.M.		3·60	1·26
10.15 A.M. to 2.15 P.M.	30	2·89	2·42
2·15 P.M. to 6.15 P.M.		3·56	2·60
7.0 P.M. to 9.30 P.M.	90	6·70	1·84
9.30 P.M. to 8.15 A.M.		6·21	1·68

[1] *Loc. cit.* [2] *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 364.

[3] Schmidt, urea, sodic chloride, and potassium nitrate, *Ann. d. Phys. u. Chem.*, Leipzig, 1861, Bd. cxiv. S. 391.

[4] Schmidt, *loc. cit.*, gum and albumin through ox-pericardium; Hoppe-Seyler, *Virchow's Archiv*, 1856, Bd. ix. S. 245, blood-serum through ureter; Runeberg, *loc. cit.*, gut, ureter, and pleural membrane, with serum, ascitic, and pleuritic fluids; Gottwalt, *loc. cit.*, egg albumin, hydrocele fluid, serum, and parovarian cyst fluid through ureter.

[5] *Loc. cit.* [6] *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 508.

On the other hand, the older experiments of Schmidt[1] with gum and albumin gave quite opposite results; thus (p. 364 of 1861 paper)—

Albumin through Ox Pericardium.

Concentration of Original Solution per Cent.	Pressure.	Per Cent. Albumin in Filtrate. / Per Cent. Albumin in Original Solution.
1·6	220 mm.	·7037
	120 mm.	·6638
3·5	220 mm.	·7050
	120 mm.	·7742

And the experiments of Gottwalt[2] and v. Regéczy[3] are in agreement with those of Schmidt.

According to Löwy,[4] who filtered serum and egg albumin solutions through pig's bladder at constant pressure, rise of temperature affects the quantity of the organic solids filtering more than the inorganic, and such slight temperature changes as from 37°·5 to 41°·5 C. have a distinct effect.

It is therefore evident that our knowledge of the phenomena of filtration through animal membranes is at present very restricted, and it is of course impossible to directly apply the results of the above observers to filtrations in the living body. No experiments, perhaps, have more clearly pointed out the difference between a dead and living filter than those of Tigerstedt and Santesson[5] with the frog's lung. A fresh frog's lung, filled with ·6 per cent. sodic chloride solution, will stand a pressure of some 13 or 14 mm. of mercury without filtering for many hours; heating in water at 54° C., or treatment with weak acetic acid, frog's bile, weak sodic hydrate, or distilled water, at once, however (presumably by killing the cells), allows filtration. Leber,[6] moreover, showed that the fresh cornea, provided the epithelium of the membrane of Descemet is intact, will stand a pressure of 200 mm. of mercury, but at once allows filtration to occur when the epithelium is removed, the tissue of the cornea itself allowing fluid to pass.

It must be confessed that experiments on living membranes (and these alone) can give any information of real value; and, furthermore, it must be remembered that filtrations in the body are, as a rule, accompanied by osmotic phenomena, since filtration must nearly always occur from one solution into another, and not into air, as in most experiments.

In concluding this article, a word must be said with regard to the theory that in some cases the cells of a part take some active part in moving solutions across membranes. So little is known of cell mechanics, that if such a process does take place we have certainly no conception of its *modus operandi*, and it is at least probable that a process considered to-day as a "vital action" may in the future become capable of a simpler explanation. Certainly, if the same solution is placed on

[1] *Loc. cit.* [2] *Loc. cit.* [3] *Loc. cit.* [4] *Loc. cit.* [5] *Loc. cit.*
[6] *Arch. f. Ophth.*, Leipzig, 1873, Bd. xix. Abth. 2, S. 125.

either side of a living membrane, and a current is found to pass from one side to the other, when the possibilities of filtration and electro-osmose are excluded, we have no physical explanation. Thus Heidenhain[1] has demonstrated that serum is absorbed by the intestine. The pressure in the gut in relation to that in the capillaries, it is true, was not measured, and the serum was not the animal's own serum, yet these objections are not of great force, especially the former, since an excess of pressure in the intestine would probably cause collapse of the capillaries or venules.[2] It is absurd to maintain that the motion of the blood in the capillaries aspirates the serum through the epithelium, because the rate of the blood stream is too slow to have any appreciable effect in this direction, and weak salt solution is moved across exsected and still living gut with equality of pressure on the two sides and no stream.[3]

This class of absorption experiment appears to be the only one in which it is justifiable to speak of "vital action," for differences in the ratios of "diffusion" of two substances into serum outside the body, and in the cavities thereof, are, *per se*, no proof of such action, since, as has been already indicated, the physical permeability of membranes differs much to one and the same substance; and again, the fact that a drug affects the rate of absorption of a substance, after exclusion of the action of that drug (if any) on the circulation, is as well (and as little) explained by stating that the permeability of the membrane is altered by its combination with the drug, as by stating that the activity of the cells is affected.

In spite of the magnificent labours of Dutrochet, Graham, Pfeffer, van 't Hoff, and Arrhenius, the enigma of the physical chemistry of protoplasm in many cases still puts a limit to the physiologist's conception of the mode of motion of fluids through the membranes and cells of the body.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lvi. S. 579.

[2] The author has repeated Heidenhain's experiment, using the animal's own serum, and measuring the pressure in the gut, and in a mesenteric vein throughout. Active absorption occurs, of the water, of the organic, and of the inorganic solids of the serum, when the pressure in the gut is far below that in a mesenteric vein, and when all the lacteals leaving the loop have been ligatured.

[3] Reid, *Brit. Med. Journ.*, London, May 28, 1892.

THE PRODUCTION AND ABSORPTION OF LYMPH.

By Ernest H. Starling.

Contents.—The Production of Lymph, p. 285—The Physical Forces concerned in the Movement of Lymph, p. 299—The Absorption of Lymph from the Connective Tissues, p. 302—On the Functions of the Lymph in the Nutrition of the Tissues, p. 310.

The Production of Lymph.

The spleen is the only part of the body where the blood comes in actual contact with the living cells of the tissue. In all other parts of the body the blood flows in capillaries with definite walls consisting of a single layer of cells, and is thus separated from the tissue elements by these walls and by a varying thickness of tissue. All the interstices of the tissues are filled with a fluid, lymph,[1] which thus acts as an intermediary between blood and tissues. The tissue spaces, which are filled with lymph, are always found in association with connective tissue. They have an incomplete lining of endothelial cells, and are connected with definite channels, lymphatics, by which any excess of fluid in the part is drained off. The lymphatics all run towards the chest, where those from the lower limbs as well as from the viscera join to form a large vessel, the *receptaculum chyli*, which is continued into the chest as the thoracic duct. This runs on the left side of the œsophagus, to open into the large veins at the junction of the left internal jugular with the subclavian vein. A small vessel on the right side drains the lymph from the right upper extremity and side of the chest.

Lymph may be collected for examination by placing a cannula in one of the main lymphatics of a limb, and inducing a flow by movements of kneading and massage, from the lymphatic duct of the neck, or from the thoracic duct. Since, moreover, the serous cavities of the pleura, peritoneum, pericardium, and tunica vaginalis are in free communication with the lymphatic system, any fluid which is normally found in them may be looked upon as lymph. The various analyses of lymph that have been made, show that its composition may vary considerably according to the locality from which it is derived and the circumstances under which it is obtained. Certain general characteristics are, however, common to all specimens of lymph. It is always slightly alkaline, and clots spontaneously at a variable time after it has left the vessels,

[1] Adler and Meltzer (*Journ. Exper. Med.*, Baltimore, 1896, vol. i. No. 3) draw a sharp distinction between the interstitial fluid of the tissue spaces, and the lymph obtained from the lymphatics which drain these spaces.

forming a colourless clot of fibrin. It contains from 2 to 8 parts per 100 of solids, of which about 1 per cent. consists of inorganic salts, while the rest is made up chiefly of proteids. The proteids are similar to those of the blood plasma; and it seems that the process of clotting is identical in the two fluids. The salts vary very little in different samples of lymph, and are generally described as being present in exactly the same proportions as in the blood plasma from which the analysed specimen of lymph was derived. Hamburger has recently called attention to the existence of minute differences of composition in the salts of the two fluids, and this difference may be credibly ascribed to chemical changes effected in the lymph by the tissues over which it has flowed. All specimens of lymph contain leucocytes, chiefly of the small uninuclear variety; these are found in greater numbers after the lymph has passed through a lymphatic gland. Further information regarding the composition of lymph will be found in the article on lymph and serous exudations (p. 181).

The similarity in composition between liquor sanguinis and lymph suggests that the latter may be regarded as part of the plasma which exudes through the capillary wall, bathes all the tissue elements, and is collected by the lymphatics into the thoracic duct to be returned again to the blood.

Forces involved in lymph production.—Older theories.—As to the forces involved in its production and the use of this fluid in the functions of the body, the most various views have been held. Asellius,[1] who discovered the lacteals in 1622, thought that these ducts carried the foodstuffs from the intestines to the liver to be there elaborated into blood. In order to explain the filling of the lacteals from the intestines, Asellius invoked the aid of the complicated mechanism which had already been imagined by Avicenna to account for the filling of the mesenteric veins. He explained the passage of chyle to the liver as due partly to the intestinal movements and partly to the suction-action of the blood vessels and of the liver itself. The chief factor however was, according to him, the suction-action exerted by the open mouths of the lacteals themselves, and he compares the latter to leeches, which suck blood from any surface to which they are applied. This theory was overthrown by Pecquet[2] by the discovery of the connection of the lacteals with the thoracic duct and through this with the venous system. The general lymphatics were discovered by Rudbeck[3] and Bartholin[4] almost simultaneously. In these authors we meet with the first conception of lymph apart from absorbed foodstuffs; moreover, Bartholin, assuming that this lymph is formed from the blood, discusses the possible ways by which the fluid could get from blood vessels to lymphatics. He thinks it possible that there may be a direct communication between lymphatics and blood vessels, but is more inclined to the view that the communication is indirect by means of the parenchyma of the organs. Failing to remark what Rudbeck had already noticed, namely, that the lymph had a salt taste, and like blood clotted spontaneously, he describes the lymph as pure water, and imagines that from the blood vessels there is a

[1] "De lactibus sive lacteis venis," Basel, 1628.
[2] "Experimenta nova anatomica," Paris, 1654.
[3] "Nova exercitatio anatomica, etc." 1653.
[4] "Vasa lymphatica nuper in animantibus inventa," Hafniæ, 1653.

transudation of water carrying solids in solution, the solids being taken up by the tissues, and the pure water which is left over returned by the lymphatics to the blood. We get here the first conception of the irrigation theory of tissue nutrition which has played so great a part in the speculations of later physiologists.

With Hunter[1] and Monro[2] we find a return to the older theory, that lymph was produced by a process of suction. This indefinite conception, however, allowed a considerable degree of individual licence as to the details of the process, and important authors, such as Hunter and Mascagni,[3] recognised the possibility of a simple transudation or filtration through the blood-vessel walls. This latter view, however, did not meet with general recognition, physiologists preferring to believe in the existence of the exhalant arteries which no one had yet seen or was ever going to see. Thus we find Bichat[4] definitely asserting the existence of "vasa exhalantia." Speaking of connective tissues, he writes: "Chaque cellule du tissue cellulaire est un réservoir intermédiare aux exhalants, qui s'y terminent, et aux absorbants qui en naissent." The absorption through the supposed open mouths of the lymphatic and lacteal vessels was attributed by most authorities of this time to capillary attraction, while the onward flow of the fluid in the lymphatics could, according to Cruickshank, only be explained as due to the vital activity of living cells or tissues. Haller describes the movement of the chyle from the intestines in exactly the same manner. Particularly ingenious is Hewson's[5] explanation of the absorption and movement of chyle in the lacteals. He shows that during life the blood vessels of the villi and in the papillæ of the skin and mucous membrane, by their turgescence, keep the orifices of the lacteals or the similar openings of the lymphatics patent, so that these are now capable of attracting like capillary tubes made of hard substances. The further movement of the chyle and lymph he ascribes to the peristaltic contraction of muscular fibres in the walls of the lacteals or lymphatics.

Views very similar to these were held by some of the most distinguished of subsequent physiologists, such as Prochaska, Fohmann, Burdach and Henle. In opposition to this mechanical theory of lymph formation, Johannes Müller,[6] having regard to the apparent power of choice possessed by the lacteals, some substances being absorbed while others were left, was inclined to ascribe at any rate the act of absorption to the vital activities of the living cells of the body.

On the discovery of endosmosis by Dutrochet,[7] many physiologists believed that at last the riddle of absorption and secretion of lymph was solved, and from this time onwards we find an invocation, generally more or less vague, of osmotic action to explain the phenomena of absorption and secretion.

Theory of Ludwig.—The beginning of the new era in the history of the physiology of lymph formation is marked by the important paper of Ludwig and Noll.[8] In consequence of experiments on

[1] Works, edited by Palmer, London, 1835, vol. iv. p. 299.
[2] "De venis lymphaticis valvulosis," 1757.
[3] "Vasorum lymphaticorum corporis humani historia et iconographia," 1787.
[4] "Anatomie générale," 1812.
[5] "A Description of the Lymphatic System, etc.," *Collected Works, Syd. Soc.*, 1846.
[6] "Elements of Physiology," Baly's trans., 1838, vol. i. p. 248.
[7] Previous article, p. 273. See also "Cyclopædia of Anat. and Phys.," art. "Endosmose."
[8] *Ztschr. f. rat. Med.*, 1850, Bd. ix. S. 52.

blood pressure, carried out by the aid of the mercurial manometer of Ludwig, these authors concluded that the chief factor in the formation of lymph was the pressure of the blood in the capillaries, and that in fact the lymph was essentially only the fluid part of blood which had filtered through the vessel wall into the surrounding tissues. On arriving in the tissues, this lymph or blood filtrate was still under a certain pressure, derived from the blood pressure, and it was this pressure which occasioned the movement of the lymph into and along the lymphatics. Ludwig concluded that the flow and composition of the lymph must be explained not only by filtration of the fluid parts of the blood, but also by processes of osmosis taking place between the tissue juices and the blood. He summarises his theory in the following words:—"The blood which is contained in the vessels must always tend to equalise its pressure and its chemical constitution with those of the extravascular fluids, which are only separated from it by the porous blood-vessel walls. If, for example, the quantity of blood in the vessels has increased, the mean blood pressure is also increased, and at once a portion of the blood is driven out into the tissues by a mere process of filtration. The same result is brought about when the constitution of the blood is altered by the absorption of food or by increased excretion by the kidneys, blood, or skin, or when the composition of the tissue fluids is altered in consequence of increased metabolic changes taking place in the tissues. In the latter case, the changes brought about in the lymph are effected by processes of diffusion." Since it is a condition of the maintenance of life that these chemical changes in the tissues should go on, and that the waste products should be continually excreted by the kidneys, lungs, and skin, there must be at the same time constant changes in the amount and composition of the lymph produced.[1]

The testing of this, the mechanical theory of lymph formation and the lineal descendant of the theory propounded two hundred years previously by Bartholin, has been the object of all subsequent investigations dealing with this question. Although we cannot claim to have arrived at a final decision on the matter, I shall endeavour to show in the following pages that the two processes—filtration and diffusion—described by Ludwig, will probably account for the lymph flow and composition in all the cases which have been sufficiently investigated.

It was shown many years ago by Magendie and others, that chemical differences between blood and lymph provoked a transference of the substance that was in excess from one side of the vessel wall to the other. Thus, if colouring matters, salts, or sugar be injected into the blood, they are very shortly afterwards found in the lymph in various parts of the body. If, on the other hand, these substances be injected into the tissue spaces or into the pleural or peritoneal cavities, their existence can very soon be detected in the blood, whence they make their way into the urine. Other instances of the extreme rapidity with which osmotic interchanges take place between the blood and lymph will be mentioned later on in dealing with the action of lymphagogues. Since these interchanges take place after the introduction of abnormal as well as normal substances into the body, we must assume the general applicability of the results, and look upon processes of diffusion or osmosis as one of the factors in regulating the composition of the lymph.

[1] "Lehrbuch der Physiologie," 1861, Aufl. 2, Bd. ii. S. 562.

Not so successful were Ludwig's attempts to demonstrate a direct relationship between blood pressure and lymph formation. According to Ludwig's hypothesis, the amount of lymph produced in any given part must be proportionate to the difference between the pressure in the capillaries and the pressure in the extravascular spaces. In most of Ludwig's earlier experiments on the subject this condition was found to hold good. On leading defibrinated blood through a limb, the lymph production in the limb was found proportional to the pressure at which the blood was led through it. In the testis Tomsa[1] showed that ligature of the pampiniform plexus caused a large increase in the lymph from this organ. Paschutin[2] and Emminghaus[3] found that, in the arm and leg, extensive ligature of the veins led to an increased lymph production. In all these cases, therefore, an augmented flow of lymph was obtained by raising the capillary pressure of the part. On the other hand, the two last-named observers were unable to prove any constant alteration of lymph production incident on vasomotor changes. Thus, in one experiment, Paschutin divided the brachial plexus of a dog and then stimulated the cut spinal cord, so that there was constriction of all the arteries of the body with the exception of those of the fore-limb under observation. Even this rise of pressure had no effect on the lymph flow from the fore-limb. A little later, Rogowicz,[4] working in Heidenhain's laboratory, repeated Emminghaus' experiments on the hind-limb with slight alterations, and found almost invariably a slight increase in the lymph after section of the sciatic nerve or in consequence of active vaso-dilatation. He proved, moreover, that the vaso-dilatation of the tongue produced by excitation of the lingual nerve was followed by an increased lymph production in the tongue, which might at times amount to an actual unilateral œdema of this organ.

Theory of Heidenhain. — In dealing with the laws affecting lymph production, we are hampered by the fact that, from the limbs of an animal at rest, there is, under normal conditions, no lymph flow at all, so that, when we wish to study the effects of our various procedures on the lymph production in the limb, we have artificially to bring about a lymph flow by kneading and massaging the limb. This fact introduces at once an arbitrary element into the observation, and Heidenhain suggested, therefore, that the best mode of investigating the truth of the filtration hypothesis would be to experiment on the lymph flow from the thoracic duct. This physiologist carried out a long research on the various conditions under which the lymph flow from the thoracic duct might be increased or diminished,[5] and came to the conclusion that the results of his experiments were irreconcilable with the filtration doctrine, and that we must assume that the cells forming the walls of the capillaries take an active part in lymph formation, *i.e.* that lymph must be looked upon as a secretion rather than as a transudation. A very similar conclusion had been previously arrived at by Tigerstedt,[6] mainly on theoretical grounds.

Heidenhain's arguments may be shortly summarised as follows:—

1. Obstruction of the thoracic aorta causes a general fall of arterial

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlvi. S. 185.
[2] *Arb. a. d. physiol. Anst. zu Leipzig*, 1873. [3] *Ibid.*, 1873.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1885, Bd. xxxvi. S. 252.
[5] *Ibid.*, 1891, Bd. xlix. S. 209.
[6] *Mitth. a. d. physiol. Inst. zu Stockholm*, 1886.

blood pressure below the obstruction. In spite of this fact, the lymph flow from the thoracic duct may in some cases be unaltered and even slightly increased.

2. Obstruction of the inferior vena cava above the diaphragm causes a general fall of blood pressure, and the intestines become apparently anæmic. The lymph flow from the thoracic duct is largely increased, and the lymph undergoes chemical changes, becoming more concentrated than it was before the obstruction. This lymph, according to Heidenhain, comes from the intestines, whereas, on obstruction of the portal vein, these organs yield an increased flow of a lymph which is less concentrated than normal and contains red blood corpuscles.

3. Heidenhain describes two classes of bodies, which on injection into the circulation increase the lymph flow from the thoracic duct.

The first class comprises bodies such as commercial peptone, watery extract of dried leeches or of crayfish. These increase the lymph and make it more concentrated. They usually cause a lowering of arterial blood pressure, although by careful injection this may be avoided.

The second class includes crystalloids such as sodium chloride, sugar, etc. Injection of concentrated solutions of these bodies into the circulation evokes an increased flow of lymph which is less concentrated than before. Some time after the injection, it is found that the lymph contains a greater percentage amount of injected substance than does the blood plasma. There may be a slight rise in the arterial pressure, but this rise is in no way proportionate to the augmentation in the lymph flow.

Since, therefore, the lymph flow may be increased without any corresponding elevation in the blood pressure, and since the amount of injected substance in the lymph may rise above that in the blood plasma, Heidenhain concludes that the processes of filtration and diffusion are incapable of accounting for the changes observed in the amount and composition of the lymph; although he does not deny that, under certain pathological conditions, such as heart disease and cirrhosis of the liver, dropsy or ascites may be and probably is conditioned by the increased intracapillary pressure acting in many cases on a capillary wall already weakened and abnormal in consequence of anæmic and diseased states of the blood.

Comparison of the theories of Ludwig and Heidenhain. — A renewed examination[1] of Heidenhain's experiments, combined with a more thorough investigation of their conditions, has persuaded me that, so far from overthrowing the filtration hypothesis, they furnish the strongest arguments which have yet been adduced in its favour. I may therefore give some account of these experiments, and show how they support Ludwig's contention with regard to the production of lymph.

Sources of the lymph investigated.—In dealing with the lymph flow from the thoracic duct, it is essential to know from what parts of the body this lymph is derived, especially since, as is well known, the lymphatics from all parts of the body, with the exception of the right upper extremity and right side of the neck, converge to pour their contents into this duct. In placing a cannula in the duct, in order to collect and measure the lymph, the ducts from the left side of the neck and left upper extremity are ligatured. From the

[1] Bayliss and Starling, *Journ. Physiol.*, Cambridge and London, vol. xvi. p. 159; Starling, *ibid.* vol. xvi. p. 224, and vol. xvii. p. 30.

hind-limbs we know that, in an animal at rest on the table, there is no lymph flow at all. Hence the sources of the lymph are confined to the trunk. We can, moreover, exclude the thorax and its contents, since ligature of the thoracic duct just above the diaphragm absolutely stops the lymph flow. Therefore, when dealing with the lymph flow from the thoracic duct, we deal only with the lymph coming from the abdominal viscera. As I shall show presently, the abdominal viscera, so far as their lymph is concerned, may be divided into two groups—(1) the viscera drained by the portal vein, and (2) the liver.

Influence of venous obstruction.—In testing the filtration hypothesis on the lymph flow, we have to investigate whether the flow is always proportional to the difference between the intra- and extracapillary pressures. We may regard the extracapillary pressure as not varying to any large extent, so that we have to see what effect is produced on the lymph by variations in the intracapillary pressure in the intestines and the liver. The simplest experiments on the subject are those in which some large vessel is obstructed. Speaking generally, we may say that obstruction of a large vein raises the pressure in the capillaries immediately behind it, whereas obstruction of an artery will diminish the pressure immediately in front of it. If, for instance, we ligature the portal vein, the arterial pressure is very little affected, while the pressure in the vein behind the ligature rises enormously. In consequence of this, there is a large rise of pressure in the capillaries of the intestines and spleen, so that the spleen swells and the intestines become black from venous congestion, hæmorrhages being produced into their mucous membrane. The effect of this ligature on the lymph flow from the thoracic duct is to increase it four or five times. The lymph also becomes bloody and its total solids are diminished. The diminution in solids is due solely to a diminution in proteids, the salts remaining the same as before; so that we have here an increased capillary pressure, causing an increased transudation of lymph containing a diminished percentage of proteid—a result which is also obtained when proteids are filtered with pressure through dead animal membranes. The presence of red blood corpuscles in the lymph is not a necessary consequence of a rise of pressure in the portal vein. If a less excessive rise of pressure be produced by ligaturing the vein, not at its entry into the liver but just below the pancreatico-duodenal vein, thus leaving a circuitous route for the blood to the liver through the anastomoses of this branch, an increased flow of lymph is produced, containing less proteids than normal lymph, but which may be quite free from red blood corpuscles.

Still more striking is the effect produced by Heidenhain's experiment of obstructing the vena cava just above the diaphragm (*i.e.* between the opening of the hepatic veins and the heart). The lymph is increased from ten to twenty fold, and it is found that the lymph obtained after the obstruction is free from red blood corpuscles and is more concentrated than normal lymph. Thus, in one experiment of this description, the lymph flow rose from 3 c.c. in the ten minutes preceding the obstruction to 25 c.c. in the ten minutes after the vein was occluded. At the same time the percentage of solids in the lymph rose from 4·8 per cent. before, to 6·6 per cent. after the obstruction.

What is the cause of this increased lymph flow and why is it more concentrated? To answer these questions we must find out first, the

source of the lymph, and secondly, the condition of the capillary pressure in the organ or organs from which the lymph is derived. We can determine the source of the lymph by a process of exclusion. Tying the kidney vessels and lymphatics has no effect on the usual consequences of obstructing the inferior vena cava. On the other hand, if we ligature the lymphatics in the portal fissure which carry off the liver lymph, we find that a subsequent obstruction has no effect on the lymph flow, or indeed, may slightly diminish it. We must conclude that the excess of lymph production consequent upon the obstruction is entirely derived from the liver, and not, as Heidenhain thought, from the intestines. The change in concentration is easily explained if we assume that, just as intestinal lymph is more concentrated (*i.e.* richer in proteids) than the lymph from the limbs, so the liver lymph is more concentrated than intestinal lymph, or than the mixed lymph obtained from the thoracic duct.

In order to answer the question as to the cause of this increased production of lymph in the liver, we must investigate the changes in the circulation brought about by the obstruction. On obstructing the inferior vena cava and recording the blood pressure in the chief vessels of the abdomen, we notice that the pressure in the aorta drops almost at once to a third of its previous height, whereas there is a very considerable rise of pressure both in the portal vein and inferior cava. It is probable that the effect of the rise of portal pressure on the intestinal capillaries is more than counterbalanced by the severe drop in arterial pressure, so that there is a fall of pressure in the intestinal capillaries. This conclusion is borne out by the fact that, if the abdomen be open, the obstruction of the inferior vena cava is seen to be at once followed by blanching of the intestines, as Heidenhain pointed out. On the other hand, the effect of the simultaneous rise of pressures in the portal vein and vena cava must be to increase the pressure in the capillaries of the liver to three or four times the normal amount. We have then, as the results of this experiment, no rise of pressure in the portal area and no increase of lymph flow from the portal area, a large rise of pressure in the hepatic capillaries and a very large increase of lymph flow from the liver.

Influence of aortic obstruction.—Another experiment, on which much stress has been laid by Heidenhain, is the one in which the descending aorta is obstructed in the thorax. The obstruction of this vessel is easily effected by passing an indiarubber balloon, tied on the end of a catheter, down the right carotid artery into the aorta just beyond the arch. The results of this obstruction on the lymph flow are somewhat variable. In most cases the lymph is diminished to one-half or one-third its previous amount; in a few cases the lymph is unaltered in quantity or even slightly increased. In all experiments the amount of proteids in the lymph is increased. Now, if we investigate the state of the circulation under these conditions, we find that obstruction of the thoracic aorta causes a very considerable fall of pressure in the aorta below the obstruction and a corresponding fall in the portal vein, whereas the pressure in the inferior vena cava is unaltered or in some cases even slightly increased. We must conclude, therefore, that in the intestinal capillaries the pressure has fallen considerably below its normal limits, while in the hepatic capillaries the pressure is very little altered or may even be

somewhat increased. Hence the only region of the body below the point of obstruction where the capillary pressure is not much diminished is the liver. Now we find that the liver is also the sole source of the lymph obtained under these circumstances. If the hepatic lymphatics be ligatured, and the thoracic aorta be then obstructed, the flow of lymph from the thoracic duct is absolutely stopped.

These three experiments show, therefore, that the lymph production in the organs of the abdomen is directly proportional to the capillary pressure in these organs, and not independent of them, as was imagined by Heidenhain.

Hydræmia and hydræmic plethora. — In another series of experiments we find, as was predicted by Ludwig (cf. p. 288), that a marked increase in the lymph flow is produced by a general rise of capillary pressure in all the organs of the abdomen. Such a general rise of capillary pressure may be brought about by the injection of large quantities of normal saline fluid into the circulation, thus causing a condition of hydræmic plethora. Under such circumstances the lymph may be increased from fifty to one hundred times in amount, and may in some cases run from the cannula in the duct in a steady stream. Now, in hydræmic plethora there are two changes in the circulation which might possibly be responsible for the increased production of lymph—first, the change in the composition of the blood, and secondly, the increased pressure in the capillaries of the abdominal viscera. We can decide which of these two factors is responsible for the increased lymph flow by a very simple experiment. Previously to injecting 300 c.c. of normal saline, we bleed the dog to 300 c.c., so that after the injection the total amount of circulating fluid is the same as at the beginning of the experiment. In this way we entirely avoid any rise of capillary pressure, while we have diluted the blood to an even greater extent than in the experiments in which hydræmic plethora was produced. The effect of such a simple hydræmia is to increase the lymph flow from 3 c.c. in ten minutes to 4 or 6 c.c. in ten minutes; whereas, if hydræmic plethora were produced, the lymph would be increased from 3 c.c. to 30, 50, or 100 c.c. in ten minutes. It is evident, therefore, that in the production of this increased lymph flow the all-important factor is the rise of capillary pressure; although the slight increase in the lymph flow observed as the result of simple hydræmia shows that, as might be expected, a watery plasma gives rise to a transudation of lymph more easily than does the normal more concentrated plasma.

Heidenhain's second class of lymphagogues.—In a precisely similar manner we may explain the mode of action of the substances which were described by Heidenhain as the second class of lymphagogues. These include bodies such as salt, sugar, potassium iodide, etc. The injection of a strong solution of dextrose (30 grms. in 30 c.c. water) into the veins of an animal causes a considerable increase in the lymph flow from the thoracic duct. The lymph at the same time becomes more watery than at the commencement of the experiment. Heidenhain ascribes this effect to a specific excitation of the secretory activities of the endothelial cells. The effect, however, can be explained in a much more simple fashion. All these solutions have an osmotic pressure which is considerably higher than that of normal blood plasma. A solution of dextrose that should be isotonic with the blood plasma would contain from 5 to 6 per cent. of this body. When we inject a solution

containing from 50 to 75 per cent. of dextrose, it will attract fluid from the tissues until its percentage is reduced to 5 or 6 per cent.; that is to say, 45 c.c. of fluid containing 30 grms. of dextrose will attract water from the tissues until its total volume is increased to 500 c.c. Of course this estimate is merely a rough approximation at the truth, since before the sugar has had time to attract all this fluid, a considerable amount of it will already have left the vessels by diffusion. As a matter of fact, however, we find that injection of a strong solution of dextrose is followed in a few minutes by a considerable dilution of the blood, caused by an increase in its volume. In some experiments of von Brasol,[1] the volume of the circulating blood was thus increased to twice or three times its previous amount; and these observations have been fully confirmed in a series of careful experiments made by J. B. Leathes[2] (Fig. 40).

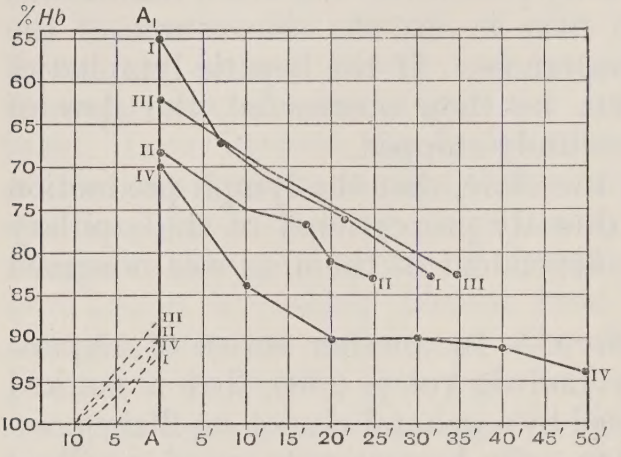

FIG. 40.—Diagram to show the dilution of the blood (*i.e.* hydræmic plethora) produced in dogs (Experiments 1, 2, 3, 4) by the injection of 5 grms. dextrose per kilo. body-weight. The ordinates represent the volume of the blood (compared with the normal) as indicated by percentage of hæmoglobin. The abscissæ represent intervals of five minutes. The line AA_1 marks the time at which the injection was finished in each experiment. The dotted lines to the left of AA_1 indicate the theoretical dilution effected by the volume of fluid injected. —After J. B. Leathes.

As we should expect, this increase in the volume of the circulating blood is attended by a large rise of capillary pressure in the abdominal viscera (Fig 41), and we have here again to decide whether it is this rise of capillary pressure, or the change in the chemical composition of the blood, that determines the increased lymph flow. This question can be solved by using the same method that we adopted when dealing with the production of the increased lymph flow in hydræmic plethora. We can entirely obviate the rise of capillary pressure if we bleed first to 300 c.c. and then inject a concentrated solution

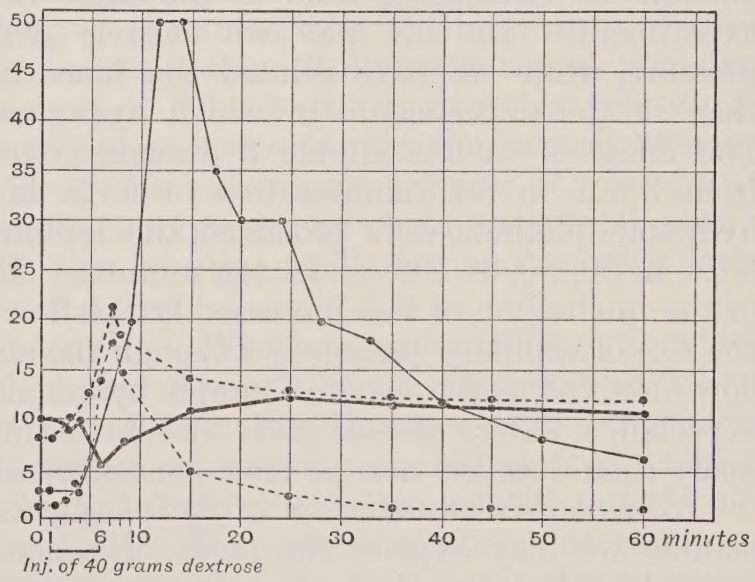

FIG. 41.—To show influence of the intravenous injection of dextrose on the blood pressure in the abdominal viscera, and on the lymph flow from the thoracic duct. The upper dotted line = pressure in portal vein. The lower dotted line = pressure in inferior vena cava. The thick continuous line = pressure in aorta. The thin continuous line = lymph flow. The ordinates represent venous pressure in centimetres of water, arterial pressure in centimetres Hg, and lymph flow in cubic centimetres per ten minutes.

[1] *Arch. f. Physiol.*, Leipzig, 1884, S. 211.
[2] *Journ. Physiol.*, Cambridge and London, 1895, vol. xix. p. 1.

containing 18 grms. of dextrose (Fig. 42). In this case the fluid that is dragged by the sugar from the tissues into the blood vessels only just suffices to make up for the previous loss of blood. No hydræmic plethora is produced; there is no rise of capillary pressure, and there is no increase in lymph flow, although an abnormally large amount of dextrose is present in the circulation.

The fact that the immediate agent in the production of the increased lymph flow is the hydræmic plethora which succeeds the injection, explains the point noticed by Heidenhain, that the efficacy of these substances is directly proportional to their attraction for water (*Wasseranziehungsvermögen*), *i.e.* to the osmotic pressure of the solution injected, and is therefore a function of their molecular weights. A similar relation was noticed by von Limbeck[1] to hold for the diuretic action of these bodies, which may therefore also be possibly determined directly by the hydræmic plethora.

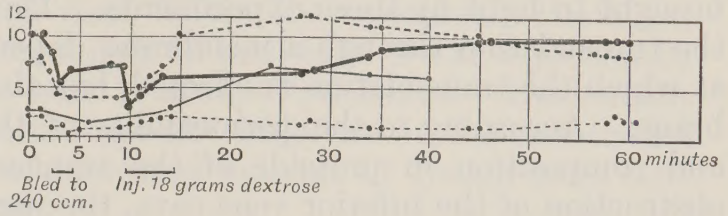

FIG. 42.—To show absence of effect of injecting dextrose after a previous bleeding. The description of the curves in Fig. 41 also applies to this figure.

The advocates of the secretory hypothesis have laid great stress on the fact that if we analyse the lymph and the blood at different periods after the injection of sugar, we find that the amount of this substance in the blood steadily diminishes (even when the kidneys are cut out of the circulation), while the sugar in the lymph gradually rises to a maximum and then diminishes parallel with but above that in the plasma. This was found to hold good for sugar by Heidenhain,[2] for potassium iodide by Ascher,[3] and for commercial peptone by myself.[4] We are not, however, justified in concluding from these facts that the sugar, etc., have been turned out from the blood vessels against pressure, so to speak. As Cohnstein[5] has pointed out, the lymph flowing at any given moment from the thoracic duct does not represent the transudation from the blood at that moment, but is derived from the lymph that has been formed some time previously. If we had a solution of sugar in gradually diminishing strength flowing into a lymphatic trunk of the leg, it is evident that this fluid would mix with the lymph in the other lymphatics, through which it flowed on its way to the thoracic duct. Later, the solution of sugar would have displaced practically all the lymph from these channels, and would flow through the thoracic duct almost undiluted. It would take, however, some considerable time to flow from the leg to the thoracic duct, so that the outflow from the duct would represent, not the fluid which was being injected into the leg at that moment, but the stronger solution which had been flowing in some time previously. If one compared, therefore, the percentage of sugar in the fluid flowing from the duct and in the fluid flowing into the leg lymphatic at different times after the beginning of the injection, we should obtain a curve exactly similar to those obtained by Heidenhain after the injection of sugar into the circulation, and regarded by him as undeniable evidence

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1888, Bd. xxv. S. 69.
[2] *Loc. cit.* [3] *Ztschr. f. Biol.*, München, 1893, Bd. xxix. S. 247.
[4] Starling, *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 131.
[5] *Arch. f. d. ges. Physiol.*, Bonn, Bd. lix. S. 350.

of secretory activity. We may conclude, therefore, that the increased flow of lymph caused by injection of the second class of lymphagogues is entirely due to the rise of capillary pressure thereby induced, and is in no wise conditioned by a stimulation of the secretory activities of the endothelial cells.

The permeability of the capillary wall.—The dependence of lymph formation on capillary pressure is not the only important relationship brought to light by these experiments. The amount and composition of the transudation through a membrane depend not only on the pressure at which the transudation is effected, but also on the nature of the membrane. According to the permeability of the membrane, so the amount and composition in proteids of the transuding fluid will vary. After obstruction of the inferior vena cava, the pressure in the intestinal capillaries, although it probably sinks below its normal height, is yet as high as that in the hepatic capillaries. Nevertheless, we get a very small amount of transudation through the intestinal capillaries, and a very large amount through the hepatic capillaries. Hence the permeability of the liver capillaries must be very much more marked than that of the intestinal capillaries. In the same way we may compare the permeability of the intestinal capillaries with those of the limb capillaries. Normally from the limb there is no flow of lymph at all, whereas a probably equal pressure in the intestinal capillaries suffices to give rise to a steady flow of lymph. If we ligature all the veins of the leg, a lymph flow may be set up, but such a flow is incomparably smaller than that produced on ligature of the portal vein. We can therefore arrange the capillaries of the body in a descending order of permeability, the liver capillaries being the most permeable and the limb capillaries the least permeable. I have already mentioned how, on filtering solutions of proteid through various membranes, the percentage of proteids in the filtrate increases with the permeability of the membrane. As we have seen, exactly the same thing holds good for the capillaries in the body. The lymph in the limbs, the filtrate through the impermeable limb capillaries, contains only from 2 to 3 per cent. proteids; that from the intestines contains from 4 to 6 per cent. proteids; while that from the permeable capillaries of the liver contains from 6 to 8 per cent. proteids—in fact, almost as much as the blood plasma itself. It is conceivable that we might alter the amount of lymph in any organ by changing, not the intracapillary pressure, but the filtering membrane, *i.e.* the endothelial wall of the capillaries. Such a change can be brought about in the body by various means. Thus the permeability of the limb capillaries is considerably increased as the effect of any local injury, such as that caused by plunging the limbs into water at 56 C. for a few minutes. Cohnheim[1] pointed out that if a cannula be placed in one of the lymphatics of the foot, and the foot be then scalded in this manner, in a few minutes the lymph begins to flow spontaneously from the cannula. The lymph which is thus produced is much richer in proteids than is lymph from a normal limb. Moreover, as Jankowski[2] showed, the amount of lymph flowing from the foot can now be varied within wide limits by altering the pressure in the capillaries, either by ligature of the vein or artery, injection of salt solution, or production of vasomotor paralysis. By this scalding, in fact, we may reduce the limb capillaries to the condition of liver capillaries.

[1] *Virchow's Archiv*, 1877, Bd. lxix. S. 516.
[2] *Ibid.*, 1883, Bd. xciii. S. 259.

Heidenhain's first class of lymphagogues.—We are now in a position to discuss the mode of action of the animal poisons included in the first class of lymphagogues. On injecting a decoction of crayfish, leeches, or mussels into the blood, the lymph flowing from the thoracic duct is increased in amount, and becomes much more concentrated than before. In both blood and lymph coagulability is lessened or abolished; the blood becomes more concentrated from a loss of plasma, while the plasma itself is less concentrated than before the injection. The blood pressure, though generally lowered, may be unaltered if the injection be carefully carried out; the heart-beat is always quickened. Heidenhain concludes that these bodies exert a specific influence on the endothelial cells, causing them to secrete an increased amount of lymph more concentrated than the blood plasma.

There can be no doubt that the greater concentration of the lymph obtained under these circumstances is due to the fact that it is chiefly derived from the liver, since the effect of these lymphagogues on the lymph flow may be almost abolished, if the portal lymphatics be ligatured previous to the injection. On investigating the changes in capillary pressure consequent on the injection, I have found that they are not sufficient to account for the increased lymph production. It is true that injection of one of these bodies is invariably followed by a considerable rise of pressure in the portal vein, associated with general vascular dilatation. But this rise of pressure is comparatively transitory (Fig. 43), lasting only fifteen to forty minutes, whereas the increased lymph flow lasts from forty minutes to two hours after the injection. Moreover, this rise of pressure in the portal vein would have more influence in increasing the capillary pressure in the intestines than in the liver. Taking these facts into consideration, we must conclude that the increased lymph flow observed after injection of lymphagogues of the first class cannot be accounted for by a rise of capillary pressure. It is open to us to conclude that these bodies act in Heidenhain's sense on the endothelial cells of the capillaries, exciting them to an active secretion. It must be remembered, however, that all these bodies are active poisons. We should expect them, therefore, to diminish rather than to excite the physiological activity of the endothelial cells. We have already seen that the effect of a slight injury to or diminished nutrition of the capillary wall is to increase its permeability. I would explain the action of these bodies, therefore, as dependent on injury to the capillary wall, and a consequent enhanced permeability, so that a pressure which is very little above the normal capillary pressure is able to cause a greatly increased transudation of fluid.

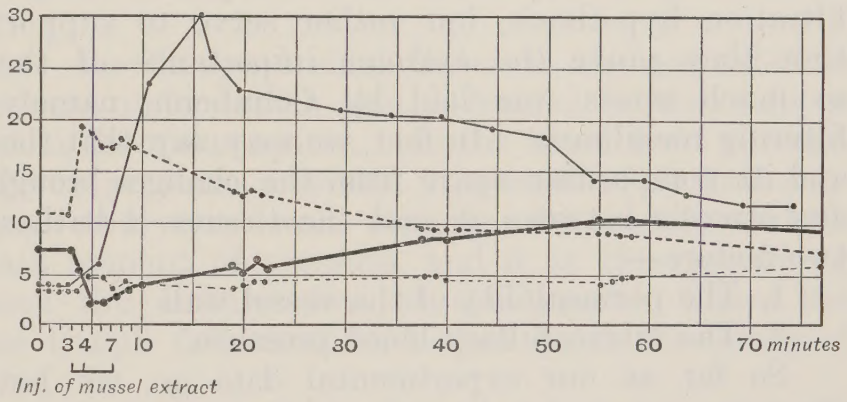

FIG. 43.—To show effects of the injection of a lymphagogue of the first class on the blood pressures in the abdominal organs, and also on the lymph flow. (For explanation of curves see Fig. 41.)

I have already mentioned that these bodies chiefly affect the capil-

laries of the liver. Their action, however, is not absolutely confined to that organ. I have experimental evidence that there is a certain degree of increased permeability of the intestinal capillaries after the injection of these lymphagogues, an increased permeability which is brought into evidence only after raising to a certain extent the pressure in these capillaries. The first class of lymphagogues also affects the capillaries of the skin. In a number of the experiments in which these bodies have been injected, we may observe a rapid development of an urticarial eruption on the skin, and it is a matter of common knowledge that the ingestion of the animals from which these bodies are derived (mussels, crayfish, lobster) is often followed in man by an eruption of urticaria which may or may not be accompanied by other symptoms of poisoning.

Another substance which seems to act directly on the capillary wall is curari. This body, however, differs from the class of lymphagogues under discussion, in the fact that its chief action is on the vessels of the limbs. The effect of curari in increasing the lymph production in the limbs was noticed long ago by Paschutin working in Ludwig's laboratory. Its direct action on the endothelial wall of the capillaries can be easily demonstrated in the living frog's web. It may be seen that, after the injection of curari, the capillary walls become apparently more sticky, so that the capillaries become filled with a number of leucocytes adhering to their walls.

Conclusions.—Thus a renewed investigation of the facts discovered by Heidenhain has shown that they are not irreconcilable with the filtration hypothesis, but rather serve to support it. At the same time they prove the extreme importance of the factor upon which so much stress was laid by Cohnheim, namely, the nature of the filtering membrane. In fact, we may say that the formation of lymph and its composition apart from the changes brought about by diffusion and osmosis between it and the tissues it bathes, depend entirely on two factors—

1. The permeability of the vessel wall.
2. The intracapillary blood pressure.

So far as our experimental data go, we have no sufficient evidence to conclude that the endothelial cells of the capillary walls take any active part in the formation of lymph. It seems rather that the vital activities of these cells are devoted entirely to maintaining their integrity as a filtering membrane, differing in permeability according to the region of the body in which they may be situated. Any injury, whether from within or without, leads to a failure of this their one function, and therefore to an increased permeability, with the production of an increased flow of a more concentrated lymph.

We have no evidence that the nervous system has any influence on the production of lymph in any part, except an indirect one by altering the capillary pressures in the part through the intermediation of vasoconstrictor or dilator fibres. This action is better marked in situations where the capillaries are normally very permeable or where the permeability has been increased by local injury to the vessels, or by the circulation of poisons in the blood stream.[1]

[1] Cf. Cohnheim u. Lassar, *Virchow's Archiv*, 1878, Bd. lxxii. S. 132; and Jankowski, *ibid.*, Bd. xciii. S. 259.

The Physical Forces concerned in the Movement of Lymph.

We may now consider briefly the forces which bring about the flow of the lymph and chyle from the origin of the lymphatics towards the termination of the thoracic duct in the subclavian vein.

In the living animal the lymphatics, like the blood vessels, are in a condition of moderate distension. The lateral pressure in the lymphatic duct of the neck was measured in 1849 by Ludwig and Noll.[1] In the dog they found that this pressure varied from 8 to 18 mm. sodium carbonate solution. A little later, Weiss[2] measured the pressure in the same vessel in the dog and horse. In the dog he found that it varied from 5 to 20 mm., and in the horse from 10 to 20 mm. soda solution. The latter observer also estimated the velocity of the lymph flow in the cervical lymphatic by means of Volkmann's hæmodromometer. He found that the average velocity was about 4 mm. in the second, a velocity which is exceedingly small as compared with the velocity of blood in arteries or veins of the same calibre, and is only a few times greater than the velocity in the capillaries. Since there is a constant flow of lymph from the periphery to the thoracic duct, it is evident that, as we trace the lymphatics towards their radicles, the pressure of the lymph must increase. This increased pressure in the peripheral parts of the lymphatic system is shown by the fact, to which Rudbeck[3] first called attention, that if a lymphatic be emptied by pressure, it always fills from the periphery, and if a ligature be placed round it, the vessel swells upon the peripheral, and shrinks on the central side of the ligature.

We see then that the first and chief factor in the onward flow of lymph is the pressure under which this is formed in the radicles of the lymphatics and in the tissue spaces. As the blood flows through the capillaries at a given pressure, a certain proportion of its fluid constituents filters through the vessel wall, forming a transudation which is still under a certain amount of pressure, and it is this remaining pressure which causes the onward flow of the lymph. Hence the ultimate cause of the lymph flow must be looked for in the energy of the heart's contraction.

When this hypothesis was first put forward by Ludwig and Noll (in opposition to the suction theories mentioned previously), it was objected to by Donders[4] on anatomical grounds. At that time it was thought that the lymphatics formed a closed system of capillaries, ramifying in the tissues; and Donders pointed out that if the pressure in the tissue juices were higher than that of the contents of the lymphatic capillaries, the effect would be, not a flow from spaces into capillaries, but a collapse of the latter with obliteration of their lumen. Further anatomical investigations have shown us, however, that, in the first place, the lymphatics are probably not a closed system of tubes, but are in communication with the tissue spaces (Recklinghausen,[5] Ludwig); and secondly, that the walls of the lymphatics, at any rate in certain situations, are so connected by strands of elastic fibres with the surrounding

[1] *Loc. cit.*

[2] "Experimentelle Untersuch. ueber die Lymphstrom," Diss., Dorpat, 1860 (quoted by Gruenhagen, Bd. i. S. 282).

[3] *Loc. cit.*

[4] *Ztschr. f. rat. Med.*, 1853, N. F., Bd. iv. S. 238.

[5] Stricker's "Histology," *Syd. Soc. Trans.*, 1869, vol. i. p. 297.

connective tissue, that a rise of tension in the meshes of the latter will only drag the walls of the lymphatics further apart, and thus increase rather than diminish their lumen.[1]

Although the blood pressure is therefore the primary mechanical factor in the movement of lymph, there are several other factors which, though subsidiary, are of considerable importance. In the first place, the flow of lymph through the thoracic duct is much aided by the respiratory movements. In all experiments on the subject of lymph formation, it is necessary to maintain the animal in as quiet a condition as possible, since any disturbance of the respiratory movements causes a variation in the lymph flow from the thoracic duct. With every inspiration, in consequence of the descent of the diaphragm, there is a rise of pressure in the abdominal cavity, and a fall of pressure in the thorax. Hence we get an emptying of the lymphatics of the abdomen, including the receptaculum chyli, and a distension of the duct in the thoracic cavity. With each expiration the thoracic duct tends to collapse to a certain degree and so empties itself into the veins, a backward flow of lymph being prevented by the valves in the duct. If a manometer be connected by a T-tube with the thoracic duct, it is found that there is a rise of pressure during expiration and a fall during inspiration, so that during the latter period the pressure may become negative.

Respiration has also an indirect influence on the lymph flow. With each inspiration the negative pressure in the thorax is increased, so that a negative pressure is also produced in the intrathoracic venous trunks, which must cause a suction of lymph through the thoracic duct into the subclavian vein. That the blood pressure in the subclavian vein at the opening of the thoracic duct is of importance for the flow of lymph, is shown by the fact that, if the pressure here is raised in any way, as by ligature of the vein, the flow of lymph is entirely stopped, and there may be a reflux of blood from the vein into the duct.

The work of Ludwig and his pupils has revealed to us the existence of certain anatomical arrangements for furthering the flow of lymph. Thus, in all tendons and aponeuroses of the body, we find a double system of lymphatics, consisting of a deep network of capillaries with meshes elongated in the direction of the fibrous bundles, and lying directly on the muscular fibres; and a superficial network with polygonal meshes lying in the peritendinous connective tissue.[2] Both networks are in connection by means of small vertical branches, and contain no valves. It is found that the slightest pressure or stretching of the aponeuroses causes a flow of lymph from the deep into the superficial meshwork, and from here into larger lymphatic vessels, which pass through the substance of the muscles to join the large lymphatic trunks. A very similar arrangement of lymphatics has been described by Ludwig and Schweigger-Seidel,[3] in the central tendon of the diaphragm. These may be injected by introducing some coloured fluid into the abdominal cavity of a freshly-killed animal, and then carrying out artificial respiratory movements.

The physiological proof of these deductions from anatomical observations was furnished by Genersich,[4] who showed that the lymph flow

[1] Gaskell, *Arb. a. d. physiol. Anst. zu Leipzig*, 1876.
[2] "Die Lymphgefässe der Fascien und Sehnen," Leipzig, 1872.
[3] *Arb. a. d. physiol. Anst. zu Leipzig*, 1866.
[4] *Ibid.*, 1870.

could be largely increased by passive flexion and extension of the limbs. We must therefore look upon the entire muscular system as one of the chief sources of the energy for maintaining the lymphatic circulation, especially as the presence of valves in the lymphatics converts every muscular contraction which may press on the vessels into a driving force.

We have finally to consider the effect of changes in the calibre of the lymphatics themselves on the onward flow of lymph. In the frog (and in other amphibia, and also in *Sauropsida*) the lymph circulation is maintained by special contractile cavities called lymph hearts, situated in pairs, an anterior pair beneath the scapulæ, and a posterior pair in the ileo-coccygeal space.

The chief points with regard to the normal anatomy and physiology of the batrachian lymph hearts have been summed up as follows, by J. Priestley :[1] —

1. The hearts are muscular sacs, the fibres of which branch and freely anastomose and are transversely striated. Their walls are penetrated by medullated and non-medullated nerve fibres, and small nerve ganglia are situated in the neighbourhood of the hearts, but no ganglion cells have as yet been recognised amidst the muscular fibres. They collect the lymph from more or less extensive lymphatic regions, and force it past valves into large veins, the anterior pair of hearts into branches of the jugular, the posterior pair into branches of the ischiatic vein. They are supplied by nerves from the spinal cord, the anterior pair by the second, the posterior pair by the tenth spinal nerve.

2. The hearts exhibit throughout life a pulsation with a mean rate of sixty to seventy a minute. It is, however, not continuously regular, being interrupted by pauses, and by periods of great acceleration. The pauses sometimes follow movements on the part of the animal, but often they cannot be set down to any definite cause. After such pauses the pulsations begin as twitches before falling into beats of normal fulness. The periods of acceleration also seem to be determined, for the most part, by movements of the animal.

3. The hearts are governed by cerebro-spinal centres—motor and inhibitory. The motor centres are situated in the spinal cord, those for the anterior pair opposite the third, and those for the posterior pair opposite the sixth vertebra. They transmit their impulses down the appropriate spinal nerves of their own side of the body; and each is independent of the rest. They originate the normal rhythm of the hearts; and their action, whatever its exact nature, is automatic, or not due directly to afferent stimuli; hence no change in the lymph current traversing the hearts can alter their rhythm. The inhibitory centre is situated in the encephalon, in the optic lobes; it is constantly in action.

4. These centres are in connection with afferent nerves. Strong stimuli, applied to the blood heart or to the abdominal viscera, lead to inhibition of the heart beats, if the upper centre is intact; while strong sensory stimuli applied to the skin may inhibit the lymph hearts whether the upper centre is present or not.

5. But though governed by the above centres, the lymph hearts seem capable of an irregular pulsation when separated from them. Such pulsation consists of flickers and indefinite confused twitchings for the most part, which, when the heart is vigorous, harmonise occasionally to full beats. The nature of these movements is still doubtful. The most that can be said about them is that they are probably not solely muscular, since curari abolishes them.

[1] *Journ. Physiol.*, Cambridge and London, 1879, vol. i. p. 1. Cf. also the account by v. Wittich in Hermann's "Handbuch," Bd. v. (2) S. 325, where full references to the literature of the subject are given.

No such mechanism exists in the mammalia. Heller and Colin have observed rhythmic contractions of the lacteals in the mesentery, but only in the herbivora. In the case of the chyle vessels, Brücke[1] has shown that the onward flow of lymph is helped by the rhythmic contractions of the muscular fibres of the intestinal villi, which empty the central cavity of the villus into the underlying network of lymphatics.

Since the walls of most lymphatic vessels and of the thoracic duct are provided with unstriated muscular fibres, we should expect these vessels to be constricted, in consequence of direct stimulation, and such constrictions have been observed in executed criminals. It has been shown more recently that an active contraction or dilatation of the lymphatics can be brought about by electrical stimulation of certain nerves. Thus Paul Bert and Laffont[2] noticed contraction of the lacteals on stimulation of the mesenteric nerves, and a dilatation of the same vessels on exciting the splanchnics. Gley and Camus[3] have lately repeated these experiments more carefully, and have obtained graphic evidence of a dilatation of the cisterna lymphatica on stimulation of the splanchnic nerve. This dilatation of the cisterna probably explains the temporary stoppage in the lymph flow from the thoracic duct which I described as the immediate effect of splanchnic stimulation.

It is probable, however, that the active contractility of the walls of the lymphatics is of very little importance for the flow of lymph through them. The only factors which are of importance are mechanical, and are—

1. The pressure under which the lymph is poured into the tissue spaces. This in its turn is dependent on the differences of pressure between the intra- and extracapillary fluids, as well as on the permeability of the vessel walls.

2. All the muscular contractions of the body, and especially those by which the respiratory movements are carried out.

The Absorption of Lymph from the Connective Tissues.

Relative importance of blood vessels and lymphatics.—Before the discovery of the lacteals by Asellius, anatomists ascribed the office of absorption generally to the veins. From this time until the beginning of the present century, no subject was more hotly disputed than the question of the relative importance of the veins and of lymphatics in the processes of absorption.

It was generally conceded that the lacteals performed practically the whole work of absorbing the products of digestion from the intestines; but the views as to the functions of the other lymphatics of the body were many and various. Thus, when Nuck[4] first made his experiments, in which he thought he injected these lymphatics from the arteries, he concluded that they had no other use than as correspondent veins, to return the lymph from such arteries as were too small to admit the red blood corpuscles. As anatomical and clinical knowledge increased, it was gradually recognised that the general lymphatics of the body had a function similar to that of the lacteals in the intestines, and like them

[1] "Ueber die Chylusgefässe und Fortbewegung des Chylus," Wien, 1853.
[2] *Compt. rend. Acad. d. sc.*, Paris, March 13, 1872.
[3] "Recherches dans les causes de la circulation lymphatique," Diss., Paris, 1894.
[4] "Adenographia curiosa," Leidæ, 1691.

were able to absorb fluids as well as solids in fine suspension or solution. A number of reasons for this conclusion are given by Johannes Müller, and I may quote some of these as an example of the arguments by which older anatomists, such as Hunter and Hewson, had come to hold this opinion. In the first place, the lymphatics often become painful, red streaks appear in their course, and the neighbouring lymphatic glands become swollen after the application by friction of irritating matters to the skin. Mascagni asserted that, in animals which died from pulmonary or abdominal hæmorrhage, the lymphatics of the pleura and peritoneum were filled with blood (Müller discredits this assertion as "extravagant"). Mascagni and Soemmering observed bile in the lymphatics coming from the liver, in cases where the bile ducts were obstructed. Tiedemann and Gmelin,[1] after tying the ductus choledochus in dogs, found the lymphatics of the liver filled with a fluid of a deep yellow colour. The lymphatic glands through which these lymphatics passed were yellow, and the yellow fluid taken from the thoracic duct contained biliary constituents. The effect of this and similar evidence on the minds of the anatomists in Hunter's time was rather curious. Since nature had provided a system—the lymphatics—on purpose to serve the office of absorption, it was considered in the highest degree improbable that this office would also be carried out by the veins, and William and John Hunter, as the result of experiments on absorption from the intestines, concluded that the veins take no part in absorption. To this view of exclusive power of absorption possessed by the lymphatics, it was objected that animals exist which possess neither lacteals nor lymphatics. It was therefore regarded as a brilliant victory for the hypothesis, when Hewson demonstrated the existence of lacteal and lymphatic vessels in birds, reptiles, and fishes.

Subsequent researches, especially by Magendie,[2] have shown, however, that absorption from all parts of the body can be effected by blood vessels as well as by lymphatics. Magendie's researches have been continued and extended of late years by Ascher[3] in the case of the connective tissues of the lower limbs, by Tubby and myself[4] in the case of the pleural and peritoneal cavities. We found, for example, that, after injecting methylene-blue or indigo-carmine into the pleura, the dye-stuff appeared in the urine within five minutes, whereas the lymph presented no trace of blue for another twenty minutes, or even two hours. It is evident that in this case the dye must have been taken up by the blood vessels and not by the lymphatics, and that this vascular absorption takes place with extreme rapidity. In a later series of experiments, Leathes[5] has shown that, after introduction of various salt solutions into the serous cavities, an interchange of constituents takes place directly between the blood and the injected fluid, so that the latter in a very short time becomes isotonic with the blood plasma. Now, in this mode of absorption by the blood vessels the so-called absorption really consists in an interchange between blood and extravascular fluids—an interchange apparently dependent entirely upon processes of diffusion between these two fluids. So long as any

[1] Quoted by Müller (Baly's translation, vol. i. p. 242).
[2] Précis élémentaire de physiologie," Paris, 1836.
[3] *Ztschr. f. Biol.*, München, 1893, Bd. xxix. S. 247.
[4] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 140.
[5] *Ibid.*, 1895, vol. xviii. p. 106.

difference in composition exists between intra- and extravascular fluids, so long will diffusion currents be set up tending to equalise this difference.

Absorption of isotonic fluids.—These experiments, therefore, have no direct bearing on the absorption of lymph, *i.e.* the normal tissue juices. In this case the fluid to be absorbed resembles in almost all particulars the blood plasma, and possesses the same osmotic pressure as the latter, so that it would seem that there are no forces of diffusion or osmosis tending to absorption. Müller[1] concludes from similar considerations that "the removal of collections of fluid must be effected in many cases by means of the lymphatics, independently of imbibition into the capillaries." The mechanism of this lymphatic absorption has been already studied. We have now to inquire whether at any time fluids, such as those normally present in the tissues and isotonic with the blood, can be taken up by the blood vessels.

We may arrange the experiments which have been made to decide this point under three headings—

1. In the first set, observations were made on the absorption of isotonic salt solutions and blood serum from the pleural and peritoneal cavities. Orlow,[2] working under Heidenhain's direction, found that such fluids were absorbed rapidly from the peritoneal cavities of living animals, while the lymph flow from a cannula placed in the thoracic duct showed no (or only slight) increase, in no way comparable to the amount of fluid absorbed. He concluded, therefore, that the absorption was effected by the blood vessels and was dependent on the vital activity of the cells lining the serous cavities or of the endothelial cells of the capillaries. Hamburger and Leathes confirmed these results, but showed that they could not depend on any vital activity of the endothelial cells, since absorption took place with equal rapidity even when poisonous solutions of sodium fluoride were employed.

The great objection to these experiments is that they do not prove conclusively absorption by the blood vessels. It is still possible that the fluids may have been taken up by the subserous lymphatic network and had not reached the thoracic duct during the experiment. This is an objection raised by Cohnstein,[3] who concludes from very similar experiments that these fluids are carried away solely by the lymphatics. It might be thought that this question could be easily decided by observing whether fluids were still absorbed from the serous cavities after ligature of both lymphatic ducts. I have made a number of experiments of this description, but have failed to get decisive results. It is true that, after ligature of both thoracic ducts as well as of the right innominate vein, isotonic salt solutions were taken up fairly quickly from the serous cavities. In none of these cases, however, could I be certain that the lymph was absolutely shut off from the blood. As a rule I injected on three succeeding days several hundred c.c. saline solution into the peritoneal cavity, the last injection containing carmine granules in suspension. On killing the dog two days after the last injection, the peritoneal cavity was generally found to be empty, and carmine granules could be traced along the glands of the anterior mediastinum, showing that, in spite of the ligature of both lymphatic ducts, there had been a

[1] *Loc. cit.* [2] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lix. S. 170.
[3] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1895, Bd. ix.

passage of lymph upwards and through the chest. We must therefore look to other methods to decide this question.

2. There is a whole series of experiments made by other observers which I think prove conclusively the power of the blood vessels to take up fluid from the tissue spaces. If an animal be bled several times, it will be found that the blood obtained in the later bleedings is more watery than that obtained at the beginning of the experiment. Now this diminution of total solids in the blood seems to be due chiefly to a dilution of the serum; the serum contains less solids than before, and is increased in volume relatively to the blood corpuscles. I may here quote some observations which show this point.[1]

Dog 11·4 kilos.—Solids of serum = 7·72 per cent. Dog then bled to 220 c.c. Thirty minutes later, solids of serum = 7·14 per cent.

In another experiment the solids of the serum were at first 6·98 per cent.; after bleeding to 200 c.c. = 6·57 per cent.; after further bleeding to 100 c.c. = 6·37 per cent.

In a smaller dog (6·5 kilos.), withdrawal of 150 c.c. blood reduced the solids of the serum from 7·77 per cent. to 6·47 per cent.

It must be noticed that this attempt to regulate the amount of the circulating blood by bringing it up to its normal volume is carried out with great rapidity, so that it is, even while an animal is being bled, found that the later portions of blood are more dilute than the earlier portions. That the fluid which is added to the blood in these cases is derived from the tissues or tissue spaces, is shown by Lazarus-Barlow's[2] experiments. This dilution of the blood takes place even when the thoracic duct is tied or when the lymph is conducted away by placing a cannula in the duct, so that it cannot be due, as was formerly thought, to an increased lymph flow into the blood.

3. In order to be absolutely certain of the power of the blood vessels to take up isotonic solutions and dropsical fluids from the tissue spaces, I carried out a series of experiments,[3] in which I led defibrinated blood through the blood vessels of amputated limbs. In each case I had a double set of transfusion apparatus, and sent one-half of the blood many times through a limb which had been rendered dropsical by the injection of isotonic salt solution, while simultaneously fluid was flowing at the same pressure through the other limb, which was not dropsical, and thus served as a control. In each case the blood was analysed and its hæmoglobin estimated before the experiment, and from both limbs after the experiment. It was invariably found that, whereas the blood which had passed from twelve to twenty-five times through the sound limb had become rather more concentrated, the blood which had passed through the œdematous limb had taken up fluid from this limb. I may here quote one of these experiments as an example:—

	Total Solids.	Percentage of Oxyhæmoglobin.
1. Blood before experiment . .	21·2 per cent.	100
2. After twenty passages through normal leg	21·4 „ „	103
3. After twenty passages through œdematous leg	20·5 „ „	95·5

[1] Tscherewkow, *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lxii. S. 304.
[2] *Journ. Physiol.*, Cambridge and London, vol. xvi. p. 13.
[3] *Ibid.*, 1895, vol. xix. p. 312.

From a consideration of these facts we must conclude that lymph and saline solutions, isotonic with the blood, may be taken up by the blood circulating through the capillaries, and that this process may occur comparatively rapidly.

Effect of intracapillary pressure.—We have already seen how any excess of intracapillary pressure, such as accompanies plethora, causes an increased transudation from the capillaries, so that the volume of circulating fluid is diminished. Now we see that, on any diminution of capillary pressure taking place, as after bleeding, the fluid in the tissue spaces goes back into the vessels to make up for the volume of circulating fluid lost. This wonderful balance between capillary pressure and lymph production or absorption is, I think, well illustrated by Lazarus Barlow's observations. This author has shown that the slight plethora produced by wrapping up a limb in Esmarch's bandage causes an appreciable increase in the transudation in other parts of the body, so that the specific gravity of the tissues of the upper limb for instance falls, while the specific gravity of the blood rises. The reverse is the case when circulation is restored to a limb which has been kept anæmic for an hour or two. Here considerable hyperæmia of the affected limb is produced, and corresponding anæmia of other parts of the body. We find, then, that absorption as well as transudation through the capillary wall is determined by the intracapillary pressure. When the pressure rises transudation is increased, when the pressure falls absorption is increased. We have seen that the dependence of transudation on capillary pressure is susceptible of a fairly simple mechanical explanation. We have now to discuss the mechanism of the absorption process.

Mechanism of absorption.—Filtration.—Is absorption effected by the active intervention of the endothelial cells, or are there physical factors at work which will serve to explain it? An explanation of absorption, which will strike anyone who investigates this problem, is that it may take place in the same manner as lymph is produced, *i.e.* by a process analogous to filtration. A series of mechanical experiments by Klemensiewicz[1] would seem at first sight to show that such a backward filtration is impossible. Klemensiewicz points out that, if fluid be passing at a given pressure through a permeable tube contained within a rigid tube, transudation will occur until the pressure of the transuded fluid is equal to that of the fluid flowing through. At a certain point in the experiment the pressure of the transuded fluid will exceed the pressure at the outflow end of the tube. The tube will collapse and the flow through it will be stopped. He imagines that the same sequence of events occurs in the living body in the presence of a considerable transudation. Arteries, capillaries, and veins are bathed in the transuded fluid. The fluid which leaves the capillaries will, if a free outflow for it be absent, after a time attain a pressure near that ruling in the capillaries and higher than the venous pressure. The veins will therefore collapse, venous obstruction will be produced, and the capillary pressure and transudation will be higher than ever, so that we have a vicious circle of events tending continually to increase the œdema of that part. Now Klemensiewicz' objections are true only under one condition—*i.e.* that the venous tubes should run freely through the lymphatic spaces of

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1881, Bd. lxxxiv.; 1886, Bd. xciv.

the tissues. If, however, we consider a system in which the inner tube is connected at various points in its circumference to the outer tube by strands of fibres, it is apparent that a rise of pressure in the space surrounding the inner tube will only serve to extend this tube still further. No collapse will take place, but a back filtration will be possible. If we cut sections of injected connective tissues, we find that the capillaries are bound to surrounding parts by radiating fibres which might possibly prevent their collapse under high extravascular pressure. In the larger veins, on the other hand, the arrangement of the fibres of the adventitia is circular and not radial, so that a high extravascular pressure would apparently cause collapse of the veins. From these anatomical facts one would conclude that a backward filtration is possible, provided that the extravascular pressure be raised in the region of the capillaries. If, however, the pressure be freely propagated through the tissues so as to affect the larger veins draining them, we shall have collapse of the veins and increased œdema. Here, as in so many other cases, we cannot get a decisive answer to our physiological questions by purely anatomical investigation, but must have recourse to physiological experiment.

The question that we have immediately to decide is, whether an increased tissue tension augments or leaves unaltered the flow of blood through the tissues, or whether it causes venous collapse and so diminishes the flow. In the former case a back filtration would be possible, and in the latter case impossible. I have investigated this point in various regions of the body, *e.g.* the connective tissues of the leg, the tongue as a type of muscular tissue, and the submaxillary gland as a type of glandular tissue. In all these cases I have found that a rise of tissue tension above the pressure in the veins causes collapse of these veins, a rise of capillary pressure, and a diminished flow of blood through the part. In these regions of the body, therefore, absorption of lymph by a backward filtration is impossible.

Imbibition.—Hamburger,[1] finding that serum and isotonic fluids are absorbed from the peritoneal cavities of animals that have been dead some hours, concludes that the life of the endothelial cell can have nothing to do with the process, and ascribes the absorption to processes of capillary and molecular imbibition, so that the absorption of fluids would be analogous to the taking up of fluids and gases by animal charcoal. Though these factors probably co-operate to a certain extent in the distribution of the fluid through the tissues surrounding the serous cavities, it is evident that they would be much more pronounced in dying and disintegrating tissues, and could with difficulty explain the taking up of fluids by the blood vessels. They would certainly not explain the wonderful balance which exists between the intracapillary pressure and the amount of fluid transuded from or absorbed by the blood vessels. What, then, is the explanation of this absorption?

Osmosis.—The explanation is, I believe, to be found in a property on which much stress was laid by the older physiologists, and which they termed the high endosmotic equivalent of albumin. It must be remembered that the older physiologists used animal membranes in their experiments on osmotic interchanges. These membranes permit the passage of water and salts, but hinder the passage of coagulable proteid. The application of semipermeable membranes to the measure-

[1] *Arch. f. Physiol.*, Leipzig, 1895, S. 281.

ment of osmotic pressure has shown that the osmotic pressures of salts and other crystalloids are enormously higher than those of colloids such as albumin, and it has therefore been supposed that the osmotic pressure of the proteids in the serum, being so insignificant, must be of no account in physiological processes. The reverse is, however, the case. Whereas the enormous pressures of the salts and crystalloids in the various fluids of the body are of very little importance for most physiological functions, the comparatively insignificant osmotic pressure of the albumins is of great importance—and for this reason. It has been shown that bodies in solution behave in most respects like gases. Now, there can be no difference in pressure between two gases in a vessel which are not separated or are only divided by a screen freely permeable to both gases. In the same way, if we have two solutions of crystallised substances separated by a membrane which offers free passage to the water and the salts on either side, there can be no enduring difference of the osmotic pressure on the two sides, especially if a free agitation of the fluids on both sides is kept up. The pressures on the two sides will be speedily equalised, and then any flow of fluid from one side to the other will cease. Now, the capillaries in the living body represent such a membrane. Leathes[1] has shown that, within five minutes after the injection of sugar or salt into the blood vessels, their osmotic pressures in the blood and lymph have become equal. Supposing, however, that we have on one side of this membrane a substance to which the membrane is impermeable, this substance will exert an osmotic pressure and will attract water from the other side of the membrane with a force proportional to its osmotic pressure. This attraction of fluid must go on until all the fluid has passed through the membrane to the side where the indiffusible substance is.

Now the capillaries of the limbs are almost impermeable to proteids. In consequence of this impermeability, the fluid which is transuded from the capillaries under pressure contains very little proteid. From what I have just said, it follows that the proteids left in solution within the capillaries must exert a certain osmotic attraction on the salt solution outside the capillaries. It is easy to measure the value of this attractive force. If we place blood serum in a small thistle funnel, over the open end of which is stretched a layer of peritoneal membrane soaked in gelatine, and immerse the inverted funnel into salt solution which is isotonic or even hypertonic as compared with the serum, within the next two days fluid will pass into the funnel and will rise in its capillary stem to a considerable height. I have found that the osmotic pressure of the non-diffusible portions of blood serum, measured in this way, may amount to about 30 mm. Hg. The importance of this fact is obvious. Although the osmotic pressure of albumin is so insignificant, it possesses an order of magnitude comparable to that of the capillary pressures; and whereas capillary pressure determines transudation, the osmotic pressure of the proteids of the serum determines absorption. Moreover, the osmotic attraction of the serum for the extravascular fluid will be proportional to the force expended in the production of this extravascular fluid, so that at any given time there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids. With increased capillary pressure we shall have increased

[1] *Journ. Physiol.*, Cambridge and London, 1895, vol. xix. p. 1.

transudation, until we get equilibrium established at a somewhat higher point, when there is a more dilute fluid in the tissue spaces, and therefore a higher absorbing force to balance the increased capillary pressure. With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid until this becomes richer in proteids, and the difference between its osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure.[1]

Here, then, we have the balance of forces necessary to explain the accurate regulation of the quantity of circulating blood according to the conditions under which the animal may be placed, and it seems unnecessary to invoke the aid of vital activity to explain the process. Certain corollaries of this mode of explanation agree well with observed facts of experiment. Thus the more impermeable the capillary the smaller will be the amount of proteid exuded with the lymph. A higher capillary pressure will therefore be needed in its production, and there will be an equally high force tending to its reabsorption. A rise of capillary pressure will only increase the amount of lymph in the extravascular spaces to a certain extent, but will at the same time cause this lymph to be more dilute, so that there will be a corresponding rise in the force tending towards absorption. In consequence of this sequence of events, considerable alterations of capillary pressure may be produced in impermeable capillaries, such as those in the limbs, without causing any appreciable increase in the lymph overflow from the limbs. On the other hand, where the capillaries are very permeable, very little pressure will be required to cause a transudation, since no work is done in the concentration of a proteid solution, and we find as a matter of fact, that capillaries where the pressure is lowest—*i.e.* in the liver—are also those which are the most permeable. Here, too, the absorbing force will be insignificant, since there is very little difference in the percentage of albumin between liver blood and liver lymph.

Moreover, since the pressure on the venous side of the capillaries is considerably less than that on the arterial side, there will be a continual exudation of a very dilute lymph from the arterial capillaries, and a re-absorption of water and salts from this lymph in the venous capillaries. The lymph, therefore, will assume a composition such that the osmotic pressure of its proteids approximates the mean capillary pressure in the part where it is formed.

This osmotic difference between blood plasma and tissue fluid will not serve to explain the absorption of proteids by the blood vessels nor the absorption of serum from the serous cavities. It is difficult, however, if not impossible, to prove that serum or proteid is absorbed by the blood vessels. In some of my transfusion experiments I have rendered a limb œdematous by means of serum, and in these cases have obtained no evidence at all of absorption by the blood vessels. There is no doubt that serum may be absorbed from the pleural and peritoneal cavities, but the absorption of these fluids is very much slower than the absorption of salt solutions, and is, in fact, so slow that the whole of it can in most cases be effected by the lymphatic channels. A slow absorption of serum from tissue spaces by means of the blood vessels is also physically possible. As the cells of the tissues feed on the proteids of the fluid,

[1] For a fuller discussion of this point, cf. *Science Progress*, London, 1896, vol. v. p. 151.

the serum will tend to become gradually weaker, so that the watery and saline constituents corresponding to the proteid used up can then be absorbed by the blood vessels in the way I have indicated.

The physical process which I have described above as causing the absorption of lymph by the blood vessels must be in action at all times in the body, and must therefore be a predominant factor in the process of absorption. I have not been able to absolutely exclude the absorption of proteids by the blood vessels, but, in the absence of direct experimental evidence that such an absorption does occur, the physical factors I have described in this chapter suffice to explain the phenomena of absorption observed both under normal and under pathological conditions.

On the Functions of the Lymph in the Nutrition of the Tissues.

The fact that the tissue cells are bathed by lymph and are separated by this fluid and by the capillary wall from the blood, shows that in all interchanges between blood and tissues the lymph must act as the medium of communication.

I have already mentioned the irrigation theory of Bartholin, according to which the nutrition of the tissues was carried out by a taking up of solids from the lymph as it left the blood vessels, so that only pure water (or water and salts—Rudbeck) was left over to be carried away by the lymphatics.

The observations of the Ludwig school on the lymph flow from the limbs, showed clearly, however, that the nutrition of the tissues could be normally carried out without any lymph flow at all. The muscles of a resting limb are taking up nourishment as well as oxygen from the blood, and giving off their waste products, carbonic acid and ammonia, although not a drop of lymph may flow from a cannula placed in a lymphatic trunk of the limb. It is evident, therefore, that to a large extent, at any rate, the giving up of nourishment by blood to tissues and the taking up of the waste products of the latter through the intermediation of the lymph, is carried out in the same way as are the gaseous interchanges—*i.e.* by a process of diffusion.

I have already mentioned the experiments which demonstrate the extreme rapidity with which diffusion takes place between the blood and the lymph, so that, as Leathes points out, the time taken for the equalisation of the constitution of the two fluids after introduction of some diffusible substance into the blood is "inappreciable." There can be no doubt that such changes are of great importance for the normal metabolism of the tissues. Thus there has been considerable discussion of late years concerning the supply of lime to the cells of the mammary gland. Heidenhain pointed out that if the lime were supplied to the cells by filtration, the whole flow from the thoracic duct would be inadequate for the purpose. His conclusion that the lymph with its constituents is therefore a secretion is, however, unnecessary. As the gland cell uses up or turns out lime into the ducts of the gland, it will take up lime from the adjoining lymph, thus lowering the partial osmotic tension of the lime in its neighbourhood. There will be, therefore, a passage of lime from blood to lymph by a process of diffusion, to supply the deficiency. No flow of lymph at all is necessary to furnish the amount of lime required by the gland cell.

The case is rather different when we come to consider the supply of proteid food to the tissues. The diffusibility of the large molecular serum proteids is so small that it may be disregarded, even in the living body with its wonderfully perfect arrangement for allowing the free contact of fluids without intermingling. Hence the only way by which the tissues can obtain their supply of proteid is from the proteid which has filtered through the vessel wall in the lymph. So far as the proteid supply to the tissues is concerned, therefore, I believe that the irrigation theory is correct, unless, indeed, we attribute to the vascular epithelium the power of actively taking up proteid and transferring it from one side of the vessel wall to the other in proportion to the needs of the tissues.

Even under the former hypothesis, however, we could not, from the amount of lymph draining away from a part, draw any conclusions as to the amount of proteid which has been supplied to the part. As I have above shown, the composition of the lymph is determined by the permeability of the wall and the mean capillary pressure. If the composition of the lymph be altered after transudation, in consequence of an active using up of the proteids of the tissue cells, the effective osmotic difference between blood and lymph will be increased, and the watery and saline constituents of the lymph will be reabsorbed until the original constitution of the lymph is restored.

We may conclude, therefore, in default of definite evidence to the contrary, that while the interchange between tissues and blood, so far as diffusible substances are concerned, is effected by *diffusion* through the medium of the lymph, the proteid supply to the cells is dependent on the amount of proteid *transuding* with the lymph.

Perhaps it is on this account—*i.e.*, increased proteid supply to the cells—that chronic inflammation or hyperæmia of any part is apt to lead to its hypertrophy. Growing tissues, as well as those in a state of repair, have delicate vessels, which probably supply a lymph much richer in proteids than is supplied to adult tissues.

CHEMISTRY OF THE DIGESTIVE PROCESSES.

By B. Moore.

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The Digestive Ferments, or Enzymes.

Organised and unorganised ferments.—Fermentation is invariably brought about, directly or indirectly, by cell life, either vegetable or animal. When the action is direct, and the chemical changes involved in the process occur only in the presence of the cell, the latter is spoken of as an *organised ferment*. When the action is indirect, and the changes are the result of the presence of a soluble material secreted by the cell acting apart from the cell, this soluble substance is termed an *unorganised ferment*, *soluble ferment*, or *enzyme*.[1]

The action of an organised ferment is intimately connected with the life of the cell, and is instantly stopped by anything which either kills the cell or temporarily arrests its activity; while that of a soluble ferment is not a vital process, but one which is purely physical or chemical in its nature. As a consequence, an organised ferment is destroyed, and its specific action stopped, by any protoplasmic poison,[2] while an unorganised ferment, provided it is not precipitated, is unaffected by such reagents.

All the differences in the mode of action of organised and unorganised ferments arise from this close connection of the organised ferment with the cell. Thus, an organised ferment, provided there is a supply of nitrogenous food at its disposal, can grow and multiply in a medium in which it is sown, while an unorganised ferment can never so increase in quantity; from this it follows that the rapidity of action of an unorganised ferment depends (within limits) on the initial quantity added, but in the case of an organised ferment the initial amount soon becomes a matter of no moment.

Organised ferments are unicellular organisms (microfungi), while the

[1] This term was first used by Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1879, N. F., Bd. i. S. 236.

[2] Such as any of those substances commonly known as antiseptics.

unorganised ferments are typically found in the secretions of specialised cells of the higher plants and animals, and take an important part in the chemical changes involved in their nutrition.

There is probably at bottom very little difference in the manner of action of cellular ferments and enzymes. From the cell substances of several bacteria, extracts have been obtained possessing the same fermentative action as the living bacteria; this indicates that in such bacteria, substances are present in the cell which act like ordinary unorganised ferments, but normally remain during the life of the cell within its substance, and perform their fermentative functions there.

A good example of such an isolation of an unorganised from an organised ferment, is afforded by that series of brilliant researches into the nature of the action of the micro-organism, *torula ureæ*, upon urine, which began with the observation that the change into ammonium carbonate was not stopped by the presence of carbolic acid in sufficient amount to paralyse the growth of the micro-organism,[1] and ended in the extraction from the bacteria of a soluble ferment, which converted urea into ammonia and carbonic acid, even in the presence of chloroform, which effectually stops all bacterial action.[2]

In a similar manner, a soluble ferment, capable of inverting cane sugar, can be extracted from yeast cells after they have been killed by the action of alcohol or ether,[3] and from certain putrefactive bacteria unorganised ferments have been obtained, possessing an action on proteids analogous to that of the proteolytic ferment of the pancreatic juice. Such intracellular soluble ferments have not been shown to exist in by far the greater number of organised ferments, but if they do so exist the only remaining difference between organised and unorganised ferments is that in the former the substance formed by the cell remains in the cell substance, and does its work there, the products of its action being poured forth as a kind of secretion or excretion, while in the latter the ferment becomes separated from the cell in a secretion, and carries out its work apart from the cell.

Most of the chemical changes involved in the digestion of the food are brought about by the presence in the digestive secretions of soluble ferments. *So that digestion might be described as the physical and chemical alteration of the foodstuffs, into forms better fitted for absorption, by the action of certain soluble ferments, the digestive enzymes.*

Attempts to isolate pure enzymes.—Many attempts have been made to isolate chemically pure enzymes, but the task is very difficult, and it is highly probable that no one has yet succeeded in obtaining a pure product.

There are two great difficulties in the way: first, our ignorance of a specific precipitant for any of the enzymes; and, secondly, the extremely small quantities in which they are present in the secretions. On account of the first, the enzyme cannot be thrown out of solution unaccompanied by other substances; on account of the second, it is not present in workable quantity, and is rapidly lost in any lengthened process of chemical manipulation. When to these disadvantages are added the non-diffusibility of the enzymes, which shuts out a means of separating them from the traces of proteid which always accompany them, and their sensitiveness to reaction and temperature, some idea is obtained of the difficulties which the problem of isolation presents.

[1] Hoppe-Seyler, *Med.-chem. Untersuch.*, Berlin, 1871, Heft 4, S. 570.
[2] Sheridan Lea, *Journ. Physiol.*, Cambridge and London, 1885, vol. vi. p. 136.
[3] Hoppe-Seyler, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1871, S. 810.

Method of mechanical precipitation.—When an indifferent precipitate is produced in a solution containing an enzyme, this is often carried out of solution with the precipitate, probably in a condition of mechanical adhesion. This observation was made by Brücke, who utilised the property to free pepsin as far as possible from other substances. The method has been extended to the preparation of purified forms of other enzymes, and, as applied by Brücke[1] to pepsin, may be quoted as an example of a general method. It is as follows:—

The mucous membrane of a pig's stomach is submitted to partial self-digestion, in water acidulated with phosphoric acid; the products of this first digestion are rejected, being too rich in products of digestion, and not containing much pepsin, which clings in great part to the mucous membrane. The residue of the mucous membrane is again digested in water made acid with phosphoric acid, and after some days is filtered from insoluble residue, and just neutralised by the addition of lime water. The insoluble calcium phosphate so precipitated carries down with it all the pepsin; it is collected on a filter paper, just dissolved by cautious addition of very dilute hydrochloric acid, filtered off, and once more precipitated by the addition of just sufficient lime water. This double precipitation is to free the pepsin of proteid, which also has the property of being mechanically carried down, though more feebly than pepsin. To this somewhat purified solution of pepsin a solution of cholesterin in four parts of alcohol and one part of ether is added. On this solution mixing with the water the cholesterin becomes insoluble, and is thrown out of solution in a finely divided condition, carrying the pepsin mechanically adhering to it just as it did to the calcium phosphate. The mixture is well shaken up, and then filtered; the precipitate is washed first with water, then with water acidulated with acetic acid, and finally with water alone. It is next, without drying, shaken up with ether, free of alcohol, but saturated with water. The ether extracts the cholesterin, while the pepsin remains in the watery layer beneath; the extraction is repeated with fresh portions of ether until all the cholesterin has been removed, and finally the watery solution containing the pepsin is filtered. In this manner a solution is obtained, which actively peptonises, but contains so little proteid as not to give many of the proteid reactions.

Method of auto-digestion.—Kühne and Chittenden[2] have combined auto-digestion with precipitation by ammonium sulphate as a means of preparing purified solutions of pepsin and trypsin. The following is an outline of their methods:—

For the preparation of pepsin the mucous membrane of a pig's stomach is taken, and allowed to undergo auto-digestion for several days, until peptonisation has far advanced, and but comparatively little albumose is left. The solution, after filtration from undigested débris of nuclein, etc., is next saturated with ammonium sulphate. The pepsin is thus completely thrown out of solution along with the albumoses; this precipitate is dissolved again, after pressing in filter paper, in dilute hydrochloric acid, and allowed to go on finishing the digestion of the albumoses for some days.

The process is repeated as often as is necessary to remove the albumose, and finally the pepsin, after being dissolved by addition of water, is freed from

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xliii. S. 601; "Vorlesungen ü. Physiologie," Wien, 1885, S. 308. See also v. Heltzl, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1864, Bd. i. S. 138.

[2] *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 428. Such a method of auto-digestion can obviously only be employed in the case of proteolytic enzymes.

ammonium sulphate by dialysis, and may be precipitated by alcohol, filtered off and dried as quickly as possible.

The preparation of a purified trypsin solution is carried out by Kühne's [1] method much on the same lines:—

The pancreas first has all its fat removed by extraction with alcohol followed by ether, after which process it forms Kühne's "pancreas powder." This is digested with five times its volume of 0·1 per cent. salicylic acid for about four hours. The residue is next digested with 0·25 per cent. sodium carbonate solution for a further period of twelve hours, and the solution is separated from the undissolved part. The two extracts are now mixed, the mixture made up with carbonate of sodium solution to a strength of 0·25 to a 0·5 per cent. carbonate, and allowed to digest at 40° C. for a week, thymol being added to prevent putrefaction (0·5 per cent.). During this time the albumoses become converted into peptones, and on saturating the cold solution, made very faintly acid with acetic acid, with ammonium sulphate, trypsin is precipitated, accompanied by traces only of unconverted albumoses. The precipitate so obtained is sufficiently pure for all digestion experiments. It contains so little accompanying albumose, that, from 10 grms. of pancreas powder, merely a thin yellowish slime is obtained on the filter paper, yet this, when taken up by 100 c.c. of 0·25 per cent. sodium carbonate solution, forms a strong digestive fluid. This gives an idea of the extreme power of the digestive ferments, and shows at the same time in what mere traces they must be present in the glands. This product may be still further purified by partially precipitating the solution obtained from it with excess of alcohol, dissolving in water, separating by dialysis the bulk of the ammonium sulphate also precipitated by the alcohol, removing the last traces of ammonium sulphate by barium carbonate, and finally precipitating as a snow-white amorphous substance by excess of alcohol.

This pure product gives all the proteid reactions (unlike Brücke's pepsin), but in spite of all the elaborate and painstaking processes used in its preparation, there is no evidence that it does not still contain traces of proteid along with trypsin; the other conclusion of course would be that trypsin is itself a proteid.

Preparation of digestive extracts.—When the object is simply to test or demonstrate the action of the enzymes, and the admixture of products of digestion formed from the gland tissue is a matter of no moment, much simpler methods of preparation may be employed than those above described.

1. In many such cases a simple extraction of the gland with water may be used, if the action is to be tested immediately.

2. A general method of obtaining digestive extracts is that first recommended by v. Wittich,[2] which consists in preparing a glycerin extract. Such an extract has the advantage of efficiency and stability. It contains a good deal of proteid, and cannot be used where the products of digestion are to be exactly studied, but for general laboratory work glycerin extracts are most convenient preparations. They are easily made, and may be preserved for years. As the glycerin only slowly extracts the enzymes, the same tissue will continue for a long time to yield fresh extracts, if fresh glycerin be added.

A glycerin extract should not be made with a quite fresh gland, but with

[1] *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1878, Bd. i. S. 222; *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1886, N. F., Bd. iii. S. 463. See also *ibid.*, 1876, N. F., Bd. i. S. 195.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 193; 1870, Bd. iii. S. 339.

a gland which has been minced up and allowed to stand for a few hours (it may be in nearly all cases made faintly acid with very dilute acetic acid to set free the zymogen as enzyme). Such a minced-up gland is rubbed up in a mortar with some clean sand, taken up with glycerin, shaken up with more glycerin (10–20 parts to 1 part of gland), and allowed to stand so until required; the process of extraction is very slow, and requires from seven to fourteen days. In the case of gastric mucous membrane, 1 part per 1000 of hydrochloric acid may be added to the glycerin.

There are many modifications of the process. v. Wittich recommends digesting the minced gland (or mucous membrane) twenty-four hours in alcohol, drying after this in the air, sifting the powder through gauze to remove coarser fragments of tissue, and extracting with glycerin. It is often recommended to filter the extract after seven to fourteen days, but this is unnecessary, as the tissue neither decomposes nor becomes digested in the glycerin, and the extract improves on keeping in contact with the tissue. The enzyme accompanied by proteid may be precipitated from a glycerin extract by the addition of absolute alcohol, and so a purer extract be obtained.

Chemical nature of enzymes.—The failure of all attempts to isolate pure enzymes necessarily deprives us of the possession of any certain knowledge of the chemical nature of these substances. Analyses of the purer preparations of the enzymes give figures *approximating* to those obtained with the various proteids; but whether or not this is due to admixture with proteid it is at present impossible to say. The behaviour of Brücke's "pure" pepsin solution goes against the supposition that this enzyme is a proteid. This solution did not give the proteid reactions, and was not precipitated by any of the proteid precipitants, save neutral and basic lead acetates and platinic chloride. These results are confirmed by Sundberg,[1] who succeeded in preparing a still more proteid-free solution, which did not even react to these reagents, and was only precipitated as a slight, pure white, flocculent precipitate, on adding five to six times its volume of absolute alcohol and allowing to stand, and yet was exceedingly active in digesting fibrin. The amount of this precipitate was much too small for analysis, and it could only be shown that it was nitrogenous, and contained a certain amount of ash. This is not quite conclusive against the proteid nature of the active substance, since, as Sundberg argues, the physiological test by digestion may be much more delicate than any of the purely chemical tests. Still, the fact that it was totally unaffected by tannic acid and precipitated by alcohol has some weight against the substance being proteid in nature; since tannic acid will show 1 part of ordinary proteid in 100,000,[2] and alcohol is by no means so delicate a proteid test. It is most probable, then, that pepsin is not a proteid; and it will subsequently be seen in the description of the other enzymes that most of these have been obtained in forms which do not yield all the proteid reactions.

The enzymes are soluble in water, from which they are precipitable by saturation with ammonium sulphate or by adding excess of alcohol.[3] Most of them are unalterable, or very slowly alterable in contact with alcohol, but pepsin is an exception, being attacked and rendered inactive if left long in contact. The enzymes are commonly said to be soluble

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 319. See also under "Ptyalin."
[2] Hofmeister, *Ztschr. f. physiol. Chem.*, Strassburg, 1878–9, Bd. ii. S. 292.
[3] These may only be particular cases of their general mechanical precipitation, whenever a precipitate is caused.

in glycerin, but it has not been shown that the dried enzymes are soluble in anhydrous glycerin; on the contrary, Kühne[1] states that pure trypsin is not soluble in strong glycerin; and it is well known that, after precipitation by alcohol from glycerin extracts, the enzymes are afterwards much less soluble in glycerin.[2]

An elevated temperature rapidly destroys all enzymes when in solution, and it is of some importance that the temperature at which they are rapidly destroyed, although it varies considerably with the reaction of the solution, lies just a little below the range at which the bulk of the proteids coagulate. In the dried condition the enzymes are much more resistant to increased temperature, and can be heated to over 100° C. for some time without losing their digestive properties on cooling and dissolving in water.

The digestive action of the enzymes is not stopped by the presence of disinfectants, such as thymol, chloroform, or salicylic acid, in quantity sufficient to stop completely the action of organised ferments, particularly that of the putrefactive bacteria.[3] This fact has been turned to account practically in conducting prolonged digestion experiments, especially when the digestive action must be allowed to proceed in alkaline solution.

Mode of action of enzymes.—The manner in which ferments bring about the changes characteristic of them is very puzzling. The enzymes are altogether unaffected by the changes which they occasion, and, provided the products of the action are not allowed to become concentrated in solution, the ferment can work on indefinitely, and a *finite* amount of ferment can convert an *infinite* amount of material. The ferment may become by dilution, or unavoidable loss in manipulation, so weak that finally its action becomes inappreciable; but before this happens it can be shown that it has converted a mass of material so many times greater than its own, that the idea that it undergoes any permanent alteration in the reaction which it induces must be abandoned. Thus, according to Hammarsten,[4] one part of rennin will curdle 400,000 to 800,000 parts of milk; while Petit[5] prepared a pepsin powder which in seven hours dissolved 500,000 times its weight of fibrin.

There are numberless examples of chemical reactions, in which only well-known and much simpler compounds take a part, of a substance inducing a chemical reaction without itself becoming altered thereby. Such a substance is called a *catalytic agent*, and the reaction a *catalysis* or *catalytic reaction*. Ferment actions are such catalytic reactions, but when we say that ferments act catalytically the problem of how they act is not by any means solved; we have merely found a name for it.

In some cases, in which the presence of a substance is essential to a certain reaction, although this substance is not finally altered thereby, there is evidence that it is altered intermediately and rechanged again back to its initial condition during the reaction.

Such a case is to be found in the action of sulphuric acid in the continuous etherification process for producing ether from alcohol. It can be shown that the sulphuric acid first combines with part of the alcohol

[1] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1876, N. F., Bd. i. S. 196.
[2] v. Wittich, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 193.
[3] Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1876, N. F., Bd. i. S. 190.
[4] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1877, Bd. vii. S. 166.
[5] *Journ. de thérap.*, Paris, 1880.

molecule, forming a substance which can be isolated, and is known as ethylsulphovinic acid; and that this compound then reacts with another molecule of alcohol, forming ether and regenerating the sulphuric acid molecule, which is then free to repeat the process, and can be made to do so indefinitely.

This action may be represented thus:—

$$(1)\ C_2H_5.O.H + \begin{matrix}H\\H\end{matrix} SO_4 = H.O.H + \begin{matrix}C_2H_5\\H\end{matrix} SO_4$$

(alcohol, sulphuric acid) — (water, ethylsulphovinic acid)

$$(2)\ \begin{matrix}C_2H_5\\H\end{matrix} SO_4 + C_2H_5.OH = \begin{matrix}H\\H\end{matrix} SO_4 + \begin{matrix}C_2H_5\\C_2H_5\end{matrix} O$$

(ethylsulphovinic acid) — (alcohol) — (sulphuric acid) — (ether)

Another good example of such an interaction is that of the alternate formation of a higher oxide of nitrogen (N_2O_3) from a lower (NO), and then the regeneration of the lower oxide, which is said to occur in the formation of English sulphuric acid; the oxygen taken up in each cycle going to form, with sulphur dioxide and water, sulphuric acid; while, as a net result, the nitric oxide remains unchanged, and may take action again and again until it is dissipated by diffusion or otherwise.[1]

Such a part the enzyme may take in a ferment action; a molecule of it may unite with a molecule of the substance undergoing digestion. Thus an unstable compound may be formed; the elements of a water molecule may combine with those of the fermentable substance, forming a new substance; while the ferment is regenerated to undergo another cycle. Of all this, however, there is no experimental evidence; there is only the analogy, and analogies are sometimes misleading.

Besides these reactions, there are others in which the action of the catalytic agent is, almost undoubtedly, merely a physical one; that is to say, in which the catalytic agent does not combine with the catalysed substance, and then become regenerated. Such an action, for example, is that of a trace of iodine in converting amorphous into red phosphorus. Here the amount of iodine required is too excessively small to suppose that it combines with phosphorus in one form and yields it up in the other. The supposition is more probable that the iodine finds the phosphorus in an unstable state, and in some fashion enables it to do that which it already has a tendency to do, namely, swing into stability. Such a reaction, only still more physical in character, is found in the case of exceedingly unstable compounds (such as detonating substances), where mere mechanical percussion, most probably by producing molecular vibration, causes a chemical reaction to take place with great rapidity. It is very likely that in many cases, especially those in which the catalytic agent is merely required to be present in traces, that there is no intermediate substance formed, and that the catalytic agent acts in a physical manner, inducing a compound already unstable to pass into a more stable condition. It is not even necessary that the substance should be unstable in the usual sense of the word, but only that the new products should be

[1] A similar oxygen-carrier, of oxygen to be used in tissue metabolism, is found in hæmoglobin, which may be looked upon as a catalytic agent, taking up oxygen, parting with it to bring about a reaction, the details of which we do not know, and so becoming regenerated and coming out of the total process unchanged.

more stable; or, in other words, that there should be energy set free in the process of change.

In ferment action, the chemical energy of the resulting products is always less than that of the substances from which they were formed; this is shown by the heats of combustion of the end products amounting to less than those of the initial products.

The action of ferments is hence in all respects analogous to that of catalytic agents; there is a passage from a less stable to a more stable condition, which is brought about by an agent which is not itself altered in the process.

The two principal hypotheses are then—(1) That the enzyme combines with the substance on which it is acting, and that the unstable compound so formed decomposes, yielding the new substance and regenerating the enzyme; (2) that the enzyme is in a state of molecular movement, which induces a molecular movement in the fermentable substance, or increases such a movement when already present, so that the molecule breaks up, over-swings, or over-vibrates as it were, into a more stable condition, so giving rise to new substances.

Nature of the chemical change.—Somewhat more is known of the nature of the chemical changes induced by the ferments than of the mode in which they bring about such changes. It is probable that in all cases ferment action is accompanied by hydrolysis, *i.e.* the taking up of the elements of water.[1] This is known with certainty to be the case in all actions of diastatic and inverting ferments, and is very probably true also for proteolytic ferments. This subject will be considered more in detail in treating of the specific action of the various enzymes on the different classes of foodstuffs; reference will only be made here to the general arguments which go to show that such a process of hydrolysis is a universal accompaniment of ferment action.

1. In many cases the composition of the products of the fermentation compared with that of the initial substance shows directly a taking up of water. In those in which this is not so, carbonic anhydride is usually one of the constituents, and if this be considered as united with the elements of a water molecule to form carbonic acid, as it probably is when formed in the reaction, water is taken up here also. In all cases, however, whether the products of the reaction directly show the taking up of water or not, the presence of water is essential to the reaction, for no ferment is known which will act otherwise than in the presence of water.

2. Again, the action of any of the ferments may be closely imitated by the action on the several fermentable or digestible materials of dilute acids or alkalies, and these are recognised throughout the domain of organic chemistry as the most powerful hydrolytic agents known.

3. It has been shown that in the case of coagulation by fibrin ferment an increase of weight of dried material takes place, probably due to the elements of water being taken up in the process. This was demonstrated by taking two equal portions of plasma, allowing one to clot and not the other, and then drying both under similar conditions, when the clotted sample was found to weigh a half per cent. more than the other.[2]

[1] Hoppe-Seyler, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 1; Nencki, *Journ. f. prakt. Chem.*, Leipzig, 1879, Bd. xvii. S. 105.

[2] Observation by A. Schmidt, communicated by G. Tamman, *Ztschr. f. physiol. Chem.*, Strassburg, 1892, Bd. xvi. S. 271.

Rate of zymolysis[1] **or enzymic action.**—The rate at which digestion goes on in any digestive fluid varies chiefly with the following conditions, namely—(1) The temperature, (2) the reaction, (3) the concentration of the products of digestion, (4) the concentration of the digestive enzyme, (5) the condition of the material to be digested.

Temperature.—The digestive enzymes are very sensitive to changes in temperature; they all act most energetically at or slightly above the body temperature. The point of greatest activity is called the optimum point; as the temperature varies, either above or below this point, the rapidity of action of the enzyme slackens; and, as the interval apart from the optimum point is increased, a point is finally reached at which the action of the enzyme is no longer appreciable. Any temperature markedly above that of optimum action slowly destroys the enzyme, and this destructive action in all cases becomes very rapid at temperatures varying (between 50° and 65° C.) with the particular ferment, the reaction of the fluid in which it is so heated, and the degree of its dilution.[2] On the other hand, low temperatures, though they slow and finally stop ferment action, do not destroy the ferment; this recovers its activity completely when the temperature is again raised, even though the temperature has been kept at −5° C. for several hours.[3]

Reaction.—The variation in chemical reaction of the fluid in which they act has a similar effect on enzymes to that of variation in temperature. For each of the digestive enzymes there is a particular reaction, and degree of that reaction, at which it acts with maximum power. A departure from this degree of acidity or alkalinity causes a more or less rapid diminution in the speed with which the enzyme acts, and a sufficient amount of departure from the optimum reaction causes the destruction of the enzyme. Some of the enzymes act in solutions of either acid, neutral, or alkaline reaction, provided always that the reaction does not stray too widely from that at which they act best; examples of such are ptyalin and trypsin. Others only act with one specific reaction, and are rapidly destroyed if the reaction changes from this. Examples of these are pepsin, only active in acid solution, and rapidly destroyed by a trace of alkalinity; and the fat-splitting ferment of the pancreas, active only in alkaline or neutral solutions, and rapidly destroyed by acid.

Accumulation of dissolved products of action.—Accumulation of the products of the action of an enzyme in the solution acts unfavourably upon its continued action, slowing and finally altogether checking it.[4] This action may be to some extent prevented by removing the products formed by dialysis, or diluting them by the addition of water. In the latter case, however, the ferment is also diluted, and in the former, since the products of digestion in most cases have no very high diffusive power, the removal is very slow and incomplete.

Removal of the digestive products by dialysis has, in addition, the disadvantage that the digestive solution is diluted by the osmosis, due to

[1] This term is that proposed by Sheridan Lea, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 254.

[2] v. Wittich, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 193; 1870, Bd. iii. S. 339.

[3] Bidder u. Schmidt, "Die Verdauungssäfte, etc."

[4] Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xliii. S. 601; "Vorlesungen," Wien, 1885, Bd. i. S. 312; Cohnheim, *Virchow's Archiv*, 1863, Bd. xxviii. S. 241; Kühne, "Lehrbuch der physiol. Chem.," 1866, S. 39, 51, 52; Sheridan Lea, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 226.

the osmotic pressure of the dissolved products. This water may, of course, be removed by subsequent evaporation at a low temperature, to avoid injuring the ferment, and again dialysing; but practically the diffusive power of the usual products of digestion is so low as to render a process of alternate dialysis and evaporation a tedious and almost impossible method of freeing the solution completely of the products of digestion. This action of the accumulated products of digestion renders all digestive experiments carried out in glass essentially different from those which go on within the alimentary canal, where the products of digestion are removed as fast as they are formed. Not only must the natural process run more quickly, but there is no reason for assuming that it will even run qualitatively along the same lines. To take as an example the tryptic digestion of proteids. There are formed, as we shall see later, as end products, certain amido-acids, and a substance known as antipeptone, but long before these products are finally reached, soluble bodies are formed which can be shown to be capable of absorption and assimilation by the epithelial cells lining the intestine.

Digestion experiments *in vitro* teach us the effects of digestion alone, sundered from its constant companion in the natural process—absorption; and no perfect method has hitherto been devised whereby the effects of these two processes working in conjunction can be demonstrated. In the animal body the pure effect of digestion and absorption cannot be observed by studying the chemical composition of the intestinal contents and that of the contents of the channels of absorption, because the products of digestion are not merely absorbed by the lining cells, but are profoundly modified by them in the process. Nor can the combined effect of digestion and absorption be studied in perfection by any known method of digestion and dialysis, because no artificial dialyser bears any but a very remote resemblance to the living intestine. A dialyser of parchment paper not only removes diffusible substances with infinite slowness compared with the intestinal epithelium,[1] but it also acts on purely physical laws, diffusion taking place at rates directly proportional to the diffusion coefficients of the substances involved; while the living epithelium takes up with great avidity soluble substances which do not diffuse at all, and absolutely refuses passage to other very diffusible substances, such as soluble salts of iron. That is to say, absorption by the cell is *selective*, being governed, indeed, by fixed and definite laws, probably purely physical and chemical at bottom, but profoundly modified by the action of living protoplasm.[2]

The effects of removal of products of digestion by dialysis has been studied by Sheridan Lea,[3] in the case of starch digestion by ptyalin, and proteid digestion by trypsin. The rapidity of dialysis was increased by mechanically raising and lowering the dialysing tube, and the rate of digestion and nature of products formed were compared with those in an exactly similar experiment arranged in a glass vessel. It was found (1) that the speed of digestion was in all cases increased, and (2) that before the process came to a standstill much more conversion took place than it was possible to attain to in glass, although complete conversion never took place in either case; these differences were in every case more marked when concentrated solutions of the material to be digested were used, showing that the slower digestion and earlier stoppage

[1] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Suppl. Heft, Bd. xliii. S. 60.
[2] For a further consideration of this subject, see "Proteid Absorption," p. 430.
[3] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 226.

in glass was due to accumulation in the solution of digested products. Similar experiments on the digestion of various forms of proteid, by pepsin and hydrochloric acid, dialysing into hydrochloric acid of equal concentration, have been made by Chittenden and Amerman,[1] who found that removal of the products of digestion did not *essentially* favour peptonisation or alter the relative amount of albumose and peptone formed.

Concentration of enzyme.—The rapidity with which zymolysis takes place naturally varies with the concentration of the enzyme in the solution, as well as with the concentration of the material to be digested, *when this is soluble.* Roberts found in the case of conversion of starch by the diastatic enzyme of the pancreas, that the amount of standard starch mucilage which can be converted in a given time and at a given temperature varies directly as the quantity of active solution employed.

Schütz[2] found in the digestion of proteid by pepsin, that when the solutions employed were sufficiently dilute, the amount of conversion was proportional to the square roots of the quantities of pepsin present. Any such rule can only hold within certain limits of concentration, a maximum being reached beyond which further concentration of the enzyme has no effect.

Methods of estimating the relative activity of digestive solutions.—As none of the enzymes have been isolated in a pure condition, it follows that there is no means of estimating the absolute amount of an enzyme in solution. This is practically never a matter of any moment, but a problem which often presents itself in practical work on digestion is that of estimating the *relative* activities of two digestive extracts.

The activity of a *diastatic* enzyme can be most accurately estimated by determining the amount of sugar (maltose) formed under given conditions in a given time by a given volume of the solution, acting on a measured volume of a standard solution of starch mucilage; this, however, is a tedious and troublesome process, and for most purposes a sufficiently accurate process is that of observing when the starch has all disappeared, as shown by the failure of the iodine reaction.

Such a method has been introduced by Roberts.[3] He varies the amount of the diastatic solution added until the "*achromic point*" is reached within a period lying between the limits of four and six minutes. This *achromic point* is that point at which the starch solution ceases to give a yellow tinge with iodine, when accordingly the solution contains only achroödextrins and maltose. Roberts defines the diastatic value of a solution (denoted by the symbol D) by the volume in cubic centimetres of a standard starch mucilage which can be converted to the *achromic point* by 1 c.c. of that solution, acting during five minutes at a temperature of 40° C.

The standard solution of starch mucilage must be prepared fresh; it is made by stirring up 5 grms. of pure potato starch with 30 c.c. of water, and pouring slowly into nearly 470 c.c. of water, which is kept boiling. The mixture is stirred and boiled for a few seconds, and finally accurately made up to 500 c.c., thus giving a standard solution (1 per cent.) of starch.

The solution of iodine used is made by diluting 1 part of the *liq. iodi* of the Pharm. Brit. with 200 parts of water.

In making a determination, one proceeds as follows:—Ten c.c. of the standard starch mucilage are diluted with distilled water to 100 c.c. and

[1] *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 483.
[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 577.
[3] Diastasimetry, In "Digestion and Diet," London, 1891, p. 68.

warmed to 40° C.; a known volume of the diastatic solution to be tested is next added, say 1 c.c., noting the time; drops of the solution are then tested from time to time, say at intervals of ten seconds, with drops of iodine on a porcelain slab until no yellow tinge is produced, and the interval of time which has elapsed is noted. By altering the amount of diastatic solution added, as a result of preliminary experiment, this time must be arranged to lie between four and six minutes; if the time is shorter than four minutes, an error of a few seconds in determining the time of conversion makes too large a percentage error, or if it be much longer than six minutes the transition is too gradual at the end for the eye to accurately catch the *achromic point*. If v be the volume in cubic centimetres of diastatic solution added, n the time to reach the achromic point in minutes, and D the diastatic value of the solution as above defined—then, $D = \frac{10}{v} \times \frac{5}{n}$

This value of D gives a measure of the activity of a given diastatic solution, in terms of a standard which can be easily reproduced at any time to measure the activity of another diastatic solution, and so comparable results may be obtained.

Various methods are in use for determining the relative activity of proteolytic solutions.

The earliest method is that first introduced by Bidder and Schmidt, and used in various modifications by other experimenters. It consists in determining the weight of proteid dissolved in equal times, by equal volumes of the digestive liquids added to equal volumes of a proper digestive medium. The method is oftenest used for relative determinations of pepsin, when the medium used is hydrochloric acid solution of 1 or 2 per mille, but it may also be used for trypsin, when $\frac{1}{4}$ per cent. sodium carbonate can be used as a medium. The digestive solutions are placed in a bath at 40° C., and when they have acquired the temperature of the bath, equal weighed portions of equally finely subdivided hard-boiled white of egg (obtained by passing through gauze netting) are added to each, and digestion allowed to proceed for the same period in each case, say twenty-four hours; the liquids are then filtered, and the residues left undigested are washed, dried, and weighed; a third equal quantity of the white of egg used is also dried and weighed without previous digestion; and from the figures so obtained the amounts of dissolved white of egg are deduced, and these are taken as representing the comparative peptonising values of the two samples.

Brücke's[1] *method.*—This method consists essentially in diluting the two proteolytic solutions to be compared with the same medium (1 per mille HCl) in two series, and then picking out those two members in each series which are most nearly equal; from the relative dilution of these two the comparative activity of the two original solutions easily follows.

Vessels.	Pepsin Solution of Acidity, 1 per Mille.	Water of Acidity, 1 per Mille.
1	16	0
2	8	8
3	4	12
4	2	14
5	1	15
6	0·5	15·5
7	0·25	15·75

[1] "Vorlesungen ueber Physiologie," Wien, 1885, Aufl. 4, Bd. i. S. 311.

Hydrochloric acid is added to the two pepsin solutions, until the acidity represents 1 grm. of hydrochloric acid per litre. These are then diluted in a series of vessels with hydrochloric acid (1 per mille) according to the foregoing scheme; the figures represent volumes, say cubic centimetres.

A corresponding series of dilutions of the second solution is also prepared, and in the vessels of both series a shred of fibrin[1] is digested for a given time. At the end of the time, correspondingly advanced specimens are picked out in the two series, especial attention being paid to the more dilute samples, which give the truer indications, and the comparative power of the two solutions easily follows. For example, if No. 3 in one series corresponds to No. 5 in the other, the latter is four times as powerful as the former; a closer approximation can then evidently be obtained by a second experiment.

Grünhagen's[2] *method.*—Fibrin is swollen out by placing it for some hours in dilute hydrochloric acid. Equal weighed portions of this swollen fibrin are placed in similar filters. Over each portion an equal volume, say 1 c.c., of the various digestive solutions to be compared are poured. Soon the fibrin begins to dissolve and drop from the funnels, dissolving in the dilute acid which had previously swollen it. From the measured amounts dropping in equal times from the different funnels, or by counting the rate of the drops, the comparative activities of the various solutions can be determined. This method evidently cannot be used for trypsin.

Grützner's[3] *method.*—Also cannot be used for trypsin, but is one of the best methods for pepsin. It is a colorimetric method, and consists in measuring the velocity with which the solution under examination dissolves fibrin stained uniformly with carmine, by means of the depth of tint imparted to the solution by the finely divided particles of carmine, which are set free in the solution at a rate proportional to that of solution of the fibrin.

The method is best carried out by comparing the depth of the tints produced at observed time intervals with those of a number of standard solutions of carmine. The methods employed in preparing the stained fibrin and these standard tints are as follows:—The fibrin is first well washed in a stream of running water accompanied by kneading,[4] and then placed for twenty-four hours in a bath of weakly ammoniacal 0·25 per cent. carmine solution,[5] the volume of staining fluid being large compared with that of the mass of fibrin to be stained, and the latter being pulled into small pieces, so as to ensure thorough and uniform staining. After staining for twenty-four hours, the fibrin is removed from the staining bath and washed well in a stream of running water until it ceases to colour it. Before using for a digestion experiment, the coloured fibrin in small pieces is immersed in about five times its volume of 0·2 per cent. hydrochloric acid for thirty to sixty minutes; this swells it up to a clot-like mass, and it is used in this condition, pieces of approximately equal size being placed in equal volumes of the various digestive fluids to be compared, contained in equal-sized test tubes.

The scale of comparison tints may be prepared by adding, in varying proportion, a glycerin solution containing one-tenth per cent. of carmine, to water in test tubes of equal size; thus, to 19·9 c.c. of water are added 0·1 c.c. of one-tenth per cent. glycerin-carmine solution; to 19·8 c.c. of water, 0·2 c.c. of the same glycerin-carmine solution; and so on, finishing with a solution

[1] Approximately of equal size; a slight difference has no appreciable effect.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 203. For a method of adopting this to experiment at body temperature, see Grützner and Ebstein, *ibid.*, 1874, Bd. viii. S. 122.

[3] *Ibid.*, 1874, Bd. viii. S. 452; "Neue Untersuch. ü. Bildung u. Ausscheid. des Pepsins," Habilitationsschrift, Breslau, 1875.

[4] It may advantageously be left in water over night to remove accompanying hæmoglobin.

[5] Prepared by dissolving 1 grm. of carmine in a small volume of dilute ammonia and making up to 400 c.c. with water; the solution should only *very* faintly smell of ammonia, and if necessary must be left exposed to the air until the odour of ammonia almost disappears.

of 19 c.c. of water and 1 c.c. of glycerin-carmine solution. In this manner ten standard tints are obtained, the values of which correspond to the numbers 1 to 10; these are mounted in a stand against a uniform white background, and are used to compare with the results of digestion, after equal intervals of time. For example, if after thirty minutes' digestion the tint of one test tube corresponds most closely to that of Standard 2, while that of another corresponds to Standard 6, the latter is three times as powerful a digestive solution as the former. The digestive solutions should be so diluted that they act somewhat slowly, because after a time a maximum tint obtains, and then the weaker digestive fluid catches up on the other; the farther apart from this maximum the measurements are taken the better. Also, if a close approximation to the *comparative amounts of pepsin* in two solutions is required, after a preliminary experiment the stronger of the two must be diluted experimentally until its action is equal to that of the other, then the proportion of dilution gives the proportionate strength in pepsin of the two solutions. This determination may be most speedily attained by making a simultaneous series of dilutions of the stronger solution, and comparing the strength of their action with that of the other solution or a series made from it.

Two tubes of equal speed of action are picked out, and from their dilutions the comparative richness in pepsin of the original fluid easily follows. Grützner's method may also be employed without a scale of standard tints, by stopping digestion after an equal period, and then diluting the stronger solution until its tint becomes equal to that of the weaker, or by carrying out two series in aliquot dilution of the two solutions to be compared, and picking out equally advanced members of the two series. In case the comparison is made with solutions of unequal power, it must be remembered that what is measured is the comparative digestive power and not the comparative strength of the solutions in pepsin, because the two are not proportional;[1] in all cases it is preferable, for accuracy, to prepare solutions from the originals of equal power, and from the amount of dilutions of these to deduce the comparative strength in pepsin of the originals, as indicated above.

Mette's method.[2]—This method is stated by Samojloff to yield exact results. It consists in filling fine glass tubes of 1 to 2 mm. in diameter with fluid white of egg, then coagulating by heat, and cutting off pieces of equal length. These are placed in the digestive solutions at body temperature, and, after the lapse of a certain interval, the length of white of egg digested off is measured, which gives a measure for the comparative activities of the two fluids.

Grützner[3] has also introduced methods for comparing the diastatic and fat-splitting powers of pancreatic extracts.

That for diastatic action closely resembles Grünhagen's method for proteolytic action. Equal volumes of 3–4 per cent. starch paste are placed on similar filters, through which they do not filter until dissolved; to each filter 0·2 to 0·3 c.c. of the extracts to be compared are next added, when solution of the starch takes place at a rate proportional to the amount of enzyme present, and a comparison of the amounts filtering through in a given time supplies a measure for the activities of the extracts.

The method of comparing the fat-splitting powers of different extracts consists in allowing the extracts to act on an emulsion in presence of litmus, and noticing the time and amount to which the latter is turned red by the acid developed. The emulsion recommended is made by mixing 10 parts of

[1] See Schütz's law, p. 322.
[2] Samojloff, *Arch. de sc. biol.*, St. Pétersbourg, 1893, tome ii. p. 707.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 293, 303.

oil of almonds, 5 parts of gum-arabic, and 35 parts of water. A solution of litmus is prepared of such concentration and reaction that it shows a violet colour when placed in test tubes about a centimetre in diameter in front of white paper; in each test tube 10 c.c. of this dilute litmus solution are placed; to each five drops of emulsion are added; then equal volumes of each of the pancreatic extracts to be compared. From the times in which an equal amount of red develops in the litmus in each case, the richness of the extracts in fat-splitting ferment may be determined. Or a series of determinations for each extract, using a varying quantity of it, may be made, and the members of each series compared.

The condition of the material to be digested has also a profound effect upon the rapidity. The factors of most moment are—

1. Whether the material is fluid or solid.

2. Whether it has previously been heated (cooked) or not. In the case of starch, previous heating and formation of a starch paste shortens the process in the ratio of hours to minutes; in the case of proteids, previous heat coagulation slows the after process of digestion.

3. Materials which must first be dissolved, and must therefore be attacked from the outside, are digested more quickly when in a finely subdivided condition.

Classification of Enzymes.

Class of Enzyme.	Name of Enzyme.	Digestive Fluid in which found.	Concise Description of Specific Action.
Diastatic	1. Ptyalin	Saliva	Convert amyloses (starches and glycogen) into dextrins, maltose, and isomaltose, accompanied by a trace of glucose.
	2. Amylopsin	Pancreatic juice	
Proteolytic	1. Pepsin	Gastric juice	Converts proteids into albumoses and peptones.
	2. Trypsin	Pancreatic juice	Converts proteids into albumoses, peptones, and amido-acids.
Fat-splitting or steatolytic	Steapsin or pialyn	Pancreatic juice	Splits up neutral fats into fatty acids and glycerin.
Coagulating	1. Rennin	Gastric juice	Coagulates milk, converting caseinogen in presence of calcium salts into casein.
	2. An unnamed ferment occurring in pancreatic juice, which also coagulates milk		

Specific action of enzymes.—The different enzymes are specific in their action; that is to say, each enzyme only acts on one class of material and acts on it in a determinate manner, producing certain specific substances as the result of that action. The table on p. 326 is a classification of the *digestive* enzymes according to their specific action.

Description of the digestive enzymes.—The digestive enzymes may be here most conveniently treated of according to their occurrence in the various digestive secretions, because of the description of the mode of their separation, where more than one is found in the same digestive fluid. Their action on the different classes of foodstuffs and the products formed thereby will be considered afterwards.

Ptyalin.—In the saliva of many animals, and especially in the herbivora, a diastatic enzyme is found, to which, soon after the discovery that saliva possessed such an action,[1] the name *ptyalin* was applied. In fishes and in cetacea no salivary glands are present,[2] and in some other animals the salivary secretion possesses no diastatic action; for example, the saliva of the dog has no diastatic action, and the same statement is made for the typical carnivora in general.[3] In man, the secretion of both the parotid and submaxillary glands has a diastatic action. At birth the ferment is only found in the parotid; it makes its first appearance in the submaxillary two months later.[4] In the horse the secretion leaves the parotid with the diastatic ferment still in the condition of a zymogen, from which the enzyme is set free by treatment with alcohol or by contact with unfiltered air.[5]

Ptyalin was first separated from saliva in an impure form by Mialhe,[6] by precipitating filtered saliva with excess of absolute alcohol. A scanty flocculent proteid precipitate is so obtained, which carries down the ptyalin mechanically. Mialhe showed that this precipitate, which was insoluble in strong alcohol, but partly soluble in water or weak alcohol, possessed when dissolved the diastatic power of the original saliva. From its supposed identity with the diastase of malt, he called it *diastase animal ou salivaire*, and used the term *ptyalin* as a synonym. It is now known that ptyalin and malt diastase, though alike in their action upon starch, are not identical. This is shown best by the difference in the reaction of the two enzymes to changes in temperature. According to Roberts,[7] saliva possesses a maximum action between the temperatures of 30° and 45° C., and, according to Kjeldahl,[8] the optimum temperature is 46° C., while the enzyme is rapidly destroyed by a temperature lying between 65° and 70° C.[9] On the

[1] Leuchs, *Arch. f. d. ges. Naturl.*, Nürnberg, 1831; Schwann, *Ann. d. Phys. u. Chem.*, Leipzig, 1836, Bd. xxxviii. S. 358.

[2] According to Krukenberg ("Grundzüge einer vergleich. Physiol. der Verdauung," 1882, S. 67), in some fishes the secretion of the mucous glands of the mouth possesses a diastatic action; the same is true of the mucous secretion of the frog's mouth.

[3] Grützner, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 285; Bunge, "Lehrbuch der physiol. Chem.," Leipzig, 1894, Aufl. 3, S. 140; Neumeister, "Lehrbuch der physiol. Chem., etc.," Jena, 1893, Th. 1, S. 122.

[4] Zweifel, "Untersuch. ueber den Verdauungsapparat. der Neugeborenen," Berlin, 1874. See also Schiffer, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 205; Korowin, *ibid.*, 1873, Bd. iii. S. 158; Bayer, *ibid.*, 1876, Bd. vi. S. 172.

[5] Goldschmidt, *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 273.

[6] *Compt. rend. Acad. d. sc.*, Paris, 1845, tome xx. pp. 654, 1483.

[7] "Digestion and Diet," London, 1891, p. 79.

[8] Abstract in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1879, Bd. ix. S. 381.

[9] Roberts, *loc. cit.*; Kühne states that saliva loses its activity at a temperature of 60° C. ("Physiol. Chem.," S. 21).

other hand, the optimum temperature for malt diastase lies at 50° to 56° C.;[1] the activity does not greatly diminish until 60° C. is passed, and then rapidly decreases and disappears, the ferment being destroyed by a temperature of 80° C. Malt diastase is also much more sensitive to the presence of salicylic acid than is ptyalin, being stopped by the presence of 0·05 per cent., while ptyalin is first affected by 0·1 per cent., and not completely stopped until a strength of 1 per cent. is reached.[2]

There is unfortunately no such certainty as to the identity or non-identity of ptyalin and amylopsin (the diastatic ferment of the pancreas), which is also called ptyalin by some authors.[3] By others, the two enzymes are accounted different, because (*a*) the pancreatic action is more intense and complete, and (*b*) there are certain differences in the products formed by the action of the two enzymes.[4] It is, however, questionable whether these effects may not be entirely produced by differences in concentration in the two cases of one and the same ferment. In their behaviour to change of temperature and reaction the two enzymes are identical; the rate of conversion of starch into other substances depends on the concentration of the enzymes in the solution; and with regard to differences in the products formed, it is not denied that in prolonged salivary digestion a small quantity of dextrose is formed, it is only claimed that larger quantities of dextrose are formed in a shorter time[5] by the action of the diastatic enzyme of the pancreas; this again is a difference in degree and not in kind, and may well be due to a difference in concentration of enzyme.

Cohnheim[6] obtained ptyalin in a purer form, that is, more free from admixed proteids, by a method closely resembling that of Brücke for pepsin, and consisting essentially in producing a precipitate of tricalcic phosphate in the saliva by the addition of phosphoric acid followed by milk of lime; this precipitates mechanically ptyalin and proteid, the ptyalin dissolves more easily than the proteid on afterwards washing the precipitate with distilled water, and may in this way be separated.

The solution so obtained was actively diastatic, but *yet gave none of the usual proteid reactions*, was not coagulated on boiling, gave no reactions with nitric acid, mercuric chloride, tannin, iodine, or acetic acid and potassium ferrocyanide. The ptyalin precipitated from it was not a pure substance, but contained chlorides and phosphates of sodium and calcium.

Excess of alcohol caused a flocky precipitate of phosphates, and an amorphous granular substance coloured yellow by iodine. Dried at a low temperature, this precipitate furnished a white powder, only slightly soluble in water, which retained its diastatic action for months.

A very active material may also be obtained by v. Wittich's method

[1] Chittenden and Martin, *Stud. Lab. Physiol. Chem.*, New Haven, 1885, vol. i. p. 117; abstract in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1885, Bd. xv. S. 263; Lintner and Eckhard, *Journ. f. prakt. Chem.*, Leipzig, 1891, N. F., Bd. xli. S. 91; Stutzer and Isbert, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 72.

[2] Jul. Müller, *Journ. f. prakt. Chem.*, Leipzig, 1875, N. F., Bd. x. S. 45.

[3] Neumeister, "Lehrbuch der physiol. Chem.," Jena, 1893, Th. 1, S. 147.

[4] See Sheridan Lea, "Chemical Basis of the Animal Body," London, 1892, p. 57.

[5] Lea, however, found no dextrose, but only maltose, in his experiments quoted on p. 394. See also Brown and Heron, *Proc. Roy. Soc. London*, 1880, No. 204, p. 393; Musculus and Gruber, *Ztschr. f. physiol. Chem.*, Strassburg, 1878–9, Bd. ii. S. 177; Musculus and v. Mering, *ibid.*, S. 403; v. Mering, *ibid.*, 1881, Bd. v. S. 185.

[6] *Virchow's Archiv*, 1863, Bd. xxviii. S. 241. Compare Sundberg's statement as to similar precipitation of pepsin by alcohol and not by tannic acid, p. 316.

of extracting the salivary glands with glycerin, precipitating the glycerin extract with excess of alcohol, washing with strong alcohol, and then extracting with water.

Effects of reaction. — A knowledge of the effects of change of reaction on the amylolytic activity of ptyalin, apart from its intrinsic interest, possesses considerable importance from the bearing it has on the natural process of digestion of starch, and for this reason probably the subject has attracted the attention of a great number of workers.[1] Ptyalin is secreted in an alkaline fluid, the saliva, and after a few seconds admixture with the food passes with it into the stomach; here its alkaline reaction is lessened by the gastric secretion, and finally replaced by an acid reaction. The amount of starch changed by the ptyalin will depend on the effect of this gradual diminution in alkalinity on its activity, and if the activity is decreased thereby, on the rate at which progress is made towards an acid reaction.

It was formerly supposed that ptyalin was only active in a fluid of alkaline reaction, that it was in consequence only active during the few seconds of mastication, while the food remained in the mouth, and was instantly destroyed on coming in contact with gastric juice. More recent observations have, however, shown that the importance of saliva as a digestive fluid is much underrated by such a view.

The diastatic action of ptyalin attains a maximum when the reaction of the fluid containing it is neutral, or even faintly acid, provided the acidity is due to acid combined with proteid. Even mere traces of *free* acid, however, lessen and rapidly destroy its activity. Sodium carbonate added to neutralised saliva decreases its activity, and in greater quantity arrests it; here, again, proteids present in solution play a protecting part, and by combining with the alkali prevent its injurious action on the ferment. A solution of ptyalin free of proteid would therefore probably act best in a neutral fluid, and would be quickly destroyed by either an acid or alkaline reaction, due to acid or alkali uncombined with proteid.[2]

The diastatic action of the saliva, therefore, continues in the stomach during and after a meal until (1) the alkali of the saliva has been neutralised, (2) the proteid present in solution has been satisfied, and (3) a trace of free hydrochloric acid remains in excess. According

[1] Jacubowitsch, Lehmann's "Zoochemie," in Gmelin's "Handbuch der Chem.," Heidelberg, 1858, Bd. viii. S. 22; Paschutin, *Arch. f. Anat. u. Physiol.*, Leipzig, 1871, S. 366; Hammarsten, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1871, Bd. i. S. 35; Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1872, Abth. 3; Watson, *Trans. Chem. Soc.*, London, 1879, p. 539; Chittenden and Griswold, *Am. Chem. Journ.*, Baltimore, 1881, vol. iii. p. 305; Falk, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1881, Bd. xi. S. 444; Langley, *Journ. Physiol.*, Cambridge and London, 1880–2, vol. iii. p. 246; Nylén, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1882, Bd. xii. S. 241; Chittenden and Ely, *Am. Chem. Journ.*, Baltimore, 1882, vol. iv.; *Journ. Physiol.*, Cambridge and London, 1882, vol. iii. p. 327; Detmar, *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vii. S. 1; Langley and Eves, *Journ. Physiol.*, Cambridge and London, 1883, vol. iv. p. 18; Chittenden and Smith, *Chem. News*, London, 1885, vol. liii.; *Stud. Lab. Physiol. Chem.*, New Haven, 1885, vol. i. p. 1; John, *Centralbl. f. klin. Med.*, Bonn, Bd. xii.; Schlesinger, *Virchow's Archiv*, 1891, Bd. cxxv. S. 146; Schierbach, *Skandin. Arch. f. Physiol.*, Leipzig, 1892, Bd. iii. S. 344; Ebstein u. Schulze, *Virchow's Archiv*, 1893, Bd. cxxxiv. S. 475.

[2] It is generally held that ptyalin acts best in neutral solution or with a faint acid reaction, due to acid combined with proteid; but there are slight differences of opinion as to where the exact optimum point lies, for which the original papers should be consulted, especially those by Langley and by Chittenden and their co-workers.

to the observations of van d. Velden[1] there is no free hydrochloric acid found in the stomach until, on an average, three-quarters of an hour after a carbohydrate meal. During this time the diastatic action of the saliva must continue, and probably during most of the interval more intensely than it would with its natural reaction. In this stage gastric juice removed by the pump possesses a diastatic action on starch, but later, when free acid is present, even when saliva is added to it, has no such power. After all the proteid present in solution in the stomach has been combined with the acid first secreted in the gastric juice, and still more acid is secreted which remains free, the ptyalin not only becomes inert, but is rapidly destroyed, and does not come into action again after the acid of the gastric juice is neutralised in the small intestine.[2]

Free organic acids also act destructively on ptyalin; the concentration of acid required is greater than in the case of hydrochloric acid, and varies with the particular acid as well as with the concentration of the ferment in the solution. Different neutral metallic salts possess different actions; some diminish the activity, such as mercuric chloride, which even in a concentration of 0·005 per cent. is sufficient to stop all action; others increase it when present in small quantity, such as magnesium sulphate up to 0·025 per cent., but have an opposite effect in greater concentration.[3] Carbolic acid does not produce much effect, digestion with 5 per cent. solution for some hours being required to destroy the ferment.[4]

Pepsin.—Pepsin is very widely distributed in the animal kingdom; it is found in the gastric juice of all vertebrates, with the possible exception of some fishes.[5] In the frog it is found chiefly in the œsophagus.[6] In the crayfish a yellowish-brown fluid is found in the mouth, of strong acid reaction, which digests fibrin readily.[7] And in many insects an acid proteolytic secretion has been observed. Similar acid proteolytic secretions are also known in the vegetable kingdom, such as those which may be obtained by stimulating the leaves of insectivorous plants.[8] Whether these acid proteolytic ferments of the invertebrates and plants are identical with pepsin is not known with certainty, but they are very similar in their action.

Pepsin is found in the stomach of the herbivora at birth, and in some other animals, including man; in others, it first appears two or three weeks after birth, as in the dog and cat.[9]

The different regions of the stomach do not, on extraction, yield

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1879, Bd. iii. S. 205.

[2] See Langley, *Journ. Physiol.*, Cambridge and London, 1882, vol. iii. p. 246; Nylén, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1882, Bd. xii. S. 241; and other authorities quoted above. Opposite results were obtained by Cohnheim, *Virchow's Archiv*, 1863, Bd. xxviii. S. 248; Schiff, "Leçons sur la digestion," tome i. p. 162; and Dufresne, *Compt. rend. Acad. d. sc.*, Paris, 1879, tome lxxxix. p. 1070.

[3] Nasse, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. xi. S. 138; Chittenden and Painter, *Stud. Lab. Physiol. Chem.*, New Haven, 1885, vol. i. p. 52.

[4] Plugge, *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 550.

[5] Hammarsten, "Lehrbuch der Physiol. Chem.," Wiesbaden, 1895, Aufl. 3, S. 234.

[6] Swiecicki, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, Bd. vi. S. 172.

[7] Hoppe-Seyler, *ibid.*, S. 170.

[8] Darwin, "Insectivorous Plants"; Goebeb and Loew, *Chem. Centr.-Bl.*, Leipzig, 1893, Bd. ii. S. 1065.

[9] Moriggia, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1876, Bd. xi. S. 455; Hammarsten, *Beitr. z. Anat. u. Physiol. als Festgabe C. Ludwig*, Leipzig, 1874, S. 116; Zweifel, "Ueber d. Verdauungsapparat der Neugeborenen," Berlin, 1874.

equal amounts of pepsin: the pyloric end always contains much less than the fundus or the cardiac end, but is never quite devoid of pepsin. It was formerly held by some observers that the pepsin found in the pyloric end was due to infiltration by the secretion from the glands of the remainder of the stomach, but the secretion obtained from pyloric fistulæ contains pepsin which can only be secreted by the glands of this region of the stomach.[1]

Effects of temperature.—Pepsin in neutral solution is destroyed by a temperature of 55° C.; in a solution containing two parts per thousand of hydrochloric acid it is not destroyed at this temperature, but is destroyed in five minutes at a temperature of 65° C. By the addition of peptones or certain salts it is so protected that it is only destroyed in an equal time by a temperature of 60° C.[2] According to v. Wittich,[3] the maximum rapidity of action is found between 35° and 50° C., and the rapidity of destruction by elevated temperature (as in the case of ptyalin) is dependent on the amount of dilution of the ferment, and the duration of the high temperature. The more dilute the pepsin solution the more quickly it is destroyed, and the lower the limit of temperature necessary. Pepsin is still faintly active at 0° C.[4]

Effects of reaction.—Pepsin is only active in acid solution; the most effective acid is hydrochloric acid, but other acids are also capable of setting it in action in varying degree. The most energetic of the other acids are nitric, lactic, and phosphoric, followed at some distance by sulphuric, acetic, oxalic, and tartaric acids. The most effective acids seem also to be those which most easily swell up fibrin. Acid sodium phosphate does not confer activity on pepsin.[5]

The amount of acidity required for optimum activity varies greatly with the form of proteid to be digested; thus Brücke[6] gives for fresh fibrin ·08 per cent., but for heat-coagulated fibrin ·12 to ·16 per cent.

Supposed compound of pepsin and hydrochloric acid.—The hypothesis has been put forward, that the pepsin and hydrochloric acid in gastric juice are united to form a loose compound "pepsin-hydrochloric acid."

There is no clear evidence in favour of the existence of such a compound. It is said to be precipitated from gastric juice by the soluble salts of lead and mercury, and to be re-obtainable unaltered from the precipitate by decomposing with sulphuretted hydrogen. But it is certain that both the acid and pepsin would be thrown down by such salts, and there is no reason to suppose that they are not thrown down separately instead of as a compound, and recovered together again on decomposing the mixed precipitate. A second argument, that the supposed compound acid can be decomposed by strong acids or alkalies, and that the pepsin so separated does not again become active on the

[1] See Ebstein and Grützner, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 210; 1873, Bd. iii. S. 169; 1874, Bd. iv. S. 236; Klemensiewicz, *ibid.*, 1875, Bd. v. S. 162; Heidenhain, *ibid.*, 1878, Bd. viii. S. 245; Klug, *ibid.*, 1894, Bd. xxiv. S. 334; Äkermann, *Skandin. Arch. f. Physiol.*, Leipzig, 1895, Bd. v. S. 134.

[2] Biernacki, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 49.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 193; 1870, Bd. iii. S. 339.

[4] Flaum, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 453.

[5] Maly, Hermann's "Handbuch," Bd. v. (2) S. 73.

[6] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1859, Bd. xxxvii. S. 131; Hammarsten gives for fibrin 0·8 to 1; for myosin, casein, and vegetable proteid, 1; for hard-boiled proteid, 2·5 parts per litre.—"Lehrbuch," Aufl. 3, S. 238.

addition of hydrochloric acid, because the compound is not re-formed, meets a simple answer in the statement that the pepsin is permanently destroyed by the strong acid or alkali used. A strong argument against any such compound is that the concentrations of different acids causing equal activity in pepsin are not proportional to the chemical equivalents of the acids, as might be expected if the acids entered into chemical combination with the pepsin.[1]

Pepsin is very rapidly destroyed by solutions of alkalies or alkaline salts.[2] The principal conditions which influence the rate of destruction of pepsin by sodium carbonate are—the strength of the solution of the alkaline salt, the time during which it is allowed to act, the temperature of the mixture, and the amount of proteid present. The mere act of neutralising an acid pepsin solution may destroy a considerable part of the pepsin. When equal volumes of a fluid containing pepsin and of a 1 per cent. solution of sodium carbonate are well mixed, the greater part of the pepsin is destroyed in fifteen seconds; in a neutralised acid extract of the gastric mucous membrane of a cat, the amount thus destroyed may be $\frac{31}{32}$ of the whole. Even very dilute sodium carbonate (·005 per cent.) will cause an appreciable destruction of pepsin in one or two hours at the body temperature, provided proteids are present in small amount only.

Proteids lessen the rate of destruction of pepsin, probably by combining with the alkali or alkaline salts, for the greater the amount of sodium carbonate present the greater must be the amount of proteid to lessen appreciably the destruction. In the presence of ·5 per cent. sodium carbonate, less than ·25 per cent. of peptone has very little effect, and even 2·5 per cent. of peptone does not prevent the greater part of the pepsin from being destroyed. Thus, in the presence of 2·5 per cent. peptone, seven-eighths of the pepsin in an extract of a cat's gastric mucous membrane may be destroyed at 17° C. by ·5 per cent. sodium carbonate in sixty seconds. Pepsin prepared from a frog is less rapidly destroyed than pepsin prepared from a mammal. Carbonic acid destroys pepsin also, but less rapidly than it destroys pepsinogen.[3]

Solutions of salts of the heavy metals weaken or entirely remove the activity of pepsin solutions, according to the amount added. This effect is probably due to the enzyme being mechanically carried down in the usual fashion by the precipitate formed between heavy metal and proteid. The neutral salts of the alkalies and alkaline earths, when added (even in small quantity) to solutions of pepsin, decrease the activity. Thus Al. Schmidt[4] found that salt-free proteid dissolved in a few seconds in salt-free pepsin solution, but on the addition of 0·5–0·6 per cent. of sodium chloride the time of solution was increased three to ten times. Hydrobromic and hydriodic acids in large doses, and to a still greater extent their potassium salts, delay peptic digestion. Sulphurous acid stops peptic digestion, but arsenious and hydrocyanic acids, except in large amounts, have little effect. Carbolic acid in small quantities has also little effect, but acts injuriously in greater concentration. Salicylic acid

[1] Davidson u. Dietrich, *Arch. f. Anat. u. Physiol.*, Leipzig, 1860, S. 688; Putzeys, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1877, Bd. vii. S. 279; Hahn, *Virchow's Archiv*, 1894, Bd. cxxxvii. S. 597.

[2] Langley, *Journ. Physiol.*, Cambridge and London, 1882, vol. iii. pp. 253, 283; Langley and Edkins, *ibid.*, 1886, vol. vii. p. 371.

[3] Quoted from Langley and Edkins, *loc. cit.*

[4] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, Bd. vi. S. 23.

was formerly credited with a powerful checking action, but, as shown by Kühne,[1] while powerful in preventing the growth of bacteria, this acid has no appreciable action, especially in small quantity, on the unorganised ferments. Pepsin is much more rapidly destroyed by standing under strong alcohol than are the other enzymes.

Anything which prevents swelling of the proteid by the acid retards the progress of peptic digestion. Brücke[2] states that fibrin tied tightly round with a thread, so that it cannot be so easily swollen by the acid, is digested much more slowly. Adding a sufficient amount of neutral salt also slows the digestion, probably from a similar cause, the salt preventing the imbibition of acid by the fibrin. The comparative slowness of digestion of heat-coagulated proteid, such as coagulated white of egg, may also be due to a like cause, for such a form of proteid does not swell up with acid. Finally, stronger acid than the optimum strength does not cause so much swelling, and this may in part be the reason of the slowing due to this cause.

Variation in rapidity with form of proteid.—The time of digestion by pepsin varies enormously with the nature and condition of the proteid to be digested; coagulated white of egg requires almost as many hours as unboiled fibrin does minutes. The comparative rate of peptonisation of coagulated and non-coagulated white of egg has been much investigated, and with varying results. According to Waurinski,[3] these variations are due to want of uniformity in the concentration of acid employed as a digesting medium; with more dilute acid the coagulated proteid is much more quickly digested, but the reverse is true when acid of greater concentration is used.

The comparative speed of peptic digestion of different kinds of proteid has, because of its practical bearing, been made the subject of much investigation.[4]

Casein is the most easily digested of all forms of proteid. Fibrin is much more quickly digested than coagulated egg-white, though, according to its state of aggregation and time of boiling, the latter shows a considerable variation. In general, proteids of animal origin are more easily digested than those of vegetables, and of the latter legumin is most easily, glutin most difficultly, digestible.[5] Jessen[6] observed that muscle fibre is more rapidly dissolved when raw than when coagulated by boiling or roasting, and that boiled milk is digested more slowly than unboiled. Beef appears to be both more easily dissolved and peptonised than fish.[7]

The conclusion ought not, however, to be too hastily drawn that those forms of proteid which are most easily dissolved by gastric juice are therefore best and most nutritious; gastric juice is not the only proteolytic fluid which acts on the food. If the food has been properly

[1] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1876, N. F., Bd. i. S. 90.

[2] "Vorlesungen," Wien, 1887, Aufl. 4, Th. 1, S. 312.

[3] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1873, Bd. iii. S. 175.

[4] Besides those quoted below, see Stutzer, *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 212; 1886, Bd. x. S. 153; 1887, Bd. xi. S. 207; 1888, Bd. xii. S. 72; Pfeiffer, *ibid.*, 1887, Bd. xi. S. 1; Wolff, *Landwirthsch. Jahrb.*, 1890, Bd. xix. S. 795; Hahn, *Virchow's Archiv*, 1894, Bd. cxxxvii. S. 597.

[5] Maly, in Hermann's "Handbuch," Bd. v. (2) S. 79.

[6] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 129. See also Bergeat, *ibid.*, 1888, Bd. xxiv. S. 139.

[7] Chittenden and Cummins, *Am. Chem. Journ.*, Baltimore, 1884, vol. vi. No. 5; Popoff, *Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xxiv. S. 524.

masticated, it is not necessary that it should be dissolved before leaving the stomach. It does not follow that the foods which are more rapidly dissolved are also more rapidly peptonised, nor, indeed, that those which are more rapidly peptonised are also more thoroughly utilised by the organism.[1]

Rennin.[2]—The presence of a milk-curdling principle in the stomach of the calf has been known for ages, but it is only within recent times that it has been shown that this action is due to the presence of a soluble ferment or enzyme.

This enzyme is present in neutral aqueous infusions of the mucous membrane of the stomach of the calf and sheep, but in the case of other mammalia, of birds and of fishes, the zymogen is more stable, and the active enzyme itself is only set free on treating a neutral infusion with acid.[3]

The presence of rennin in the stomachs of birds and fishes is very remarkable, and points to some wider function of the enzyme, at present unknown to us, since it cannot be supposed that in such animals the ferment plays any part in connection with the clotting of milk. Many plant juices also contain enzymes which coagulate milk, such as the latter of the fig tree,[4] and of *Carica pepaya*, and the flowers of many cynaria. Milk[5] is also coagulated by bacterial action with the development of an acid reaction due to lactic acid (in the souring of milk). A curdy precipitate somewhat resembling a clot is caused by the addition of acids to milk, which led to the erroneous analogy being drawn, that the coagulation of milk by rennet was also an acid action, due to lactic acid set free from the lactose of the milk by ferment action.

The following is a summary of the proofs that milk coagulation is not an acid action, but due to a specific enzyme (rennin), which acts on a proteid (caseinogen) of the milk:—1. When a neutral solution of rennin (rennet) is added to alkaline milk, and the mixture is kept at 38°–40° C., complete coagulation occurs in 4–10 minutes, *and in the process the reaction remains unchanged.* 2. Solutions of caseinogen prepared from milk *and free from lactose* coagulate in presence of calcium salts, on the addition of rennin. 3. Purified solutions of rennin have no action whatever on lactose.[6]

Rennin is always present under normal conditions in human gastric juice, both at birth and in the adult.[7] The distribution of the enzyme and its zymogen in the gastric mucous membrane is similar to that of

[1] See "Absorption of Proteids," p. 441.

[2] The name is due to Sheridan Lea; that of chymosin has been proposed by Deschamp.

[3] Hammarsten, "Lehrbuch d. physiol. Chem.," Wiesbaden, 1895, Aufl. 3, S. 241.

[4] This also contains a proteolytic ferment, active in either alkaline, neutral, or acid reaction (Baginsky, *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vii. S. 209; *Arch. f. Anat. u. Physiol.*, Leipzig, 1883, S. 276).

[5] For the chemistry of milk, see p. 125.

[6] These proofs are due to: Heintz, *Journ. f. prakt. Chem.*, Leipzig, 1872, N. F., Bd. vi. S. 374; Hammarsten, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 118; 1874, Bd. iv. S. 135; 1887, Bd. vii. S. 158; "Zur Kenntniss des Caseins und der Wirkung des Labfermentes," Upsala, 1877; Al. Schmidt, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, Bd. iv. S. 154. From the fact that rennet when impure acts on lactose, but not after purification, Hammarsten supposed that gastric juice contained a third enzyme, which acted on lactose, forming lactic acid, but this has not been substantiated.

[7] Zweifel, *Centralbl. f. d. med. Wissensch.*, Berlin, 1874, Bd. xii. S. 939; Hammarsten, *Beitr. z. Anat. u. Physiol. als Festgabe C. Ludwig*, Leipzig, 1874; Schumberg, *Virchow's Archiv*, 1884, Bd. xcvii. S. 260; Boas, *Centralbl. f. d. med. Wissensch.*, Berlin, 1887, Bd. xxv. S. 417.

pepsin; that is, the pyloric part furnishes very weak extracts compared with those yielded by the fundus.[1]

Solutions of rennin, commonly called rennets, may be prepared by extracting the mucous membrane of the stomach in various ways, of which the following is a summary:—

1. Extraction of the mucous membrane of the stomach of the calf for some days with glycerin. Purer solutions may be afterwards obtained by precipitating the glycerin extract with excess of alcohol, filtering, and treating the precipitate with water.
2. Digesting the mucous membrane of the stomach for twenty-four hours at atmospheric temperature with water containing 1 to 2 parts per mille of hydrochloric acid, filtering, and neutralising.
3. Extracting with a saturated aqueous solution of salicylic acid, precipitating by excess of alcohol, and extracting the precipitate with water.
4. The best extractive for making permanent preparations is solution of sodium chloride of from 5 to 15 per cent. concentration, putrefaction being prevented by the addition of alcohol, thymol, or some such innocuous preservative.

Effects of temperature.—Rennin is quickly destroyed in neutral solution by a temperature of 70° C., in acid solution by a temperature of 63° C. The temperature of maximum activity lies at 38 to 40° C. It also acts, though more slowly, at atmospheric temperatures.

Action of acids and alkalies.—Rennin is rapidly destroyed by caustic alkalies; even 0·025 per cent. of caustic soda suffices, at atmospheric temperature in twenty-four hours, to completely destroy a very active solution. The amount of ferment so destroyed varies as usual with the duration of the action, the temperature, and the concentration of the destructive agent. In their behaviour towards alkaline carbonates rennin and its zymogen closely resemble pepsin and pepsinogen; rennin being quickly destroyed by 0·5 to 1·0 per cent. of sodium carbonate (Na_2CO_3), while its zymogen is much less readily affected thereby.[2]

Rennin is destroyed by standing under alcohol, but this change occurs more slowly than the corresponding one in the case of pepsin.

Separation of pepsin and rennin.—For the preparation of a pepsin solution free from rennin, a gastric extract containing both enzymes is submitted to digestion in 0·3 per cent. hydrochloric acid for forty-eight hours at 38° to 40° C.; the rennin is completely destroyed. Hammarsten[3] utilises Brücke's principle of mechanical precipitation, for the preparation of a rennin solution free from pepsin, in the following method. An acid infusion of the gastric mucous membrane is neutralised with magnesium carbonate, and enough neutral acetate of lead is added to *completely* precipitate all the pepsin accompanied by a portion of the rennin.[4] The filtrate is further precipitated by more lead acetate aided by ammonia, and the precipitate is separated and decomposed by *very* dilute sulphuric acid, so yielding a solution of rennin almost free from proteid. This solution is then further purified by mechanical precipitation with cholesterin.

The final product so obtained produced no effect on a flock of fibrin

[1] Hammarsten, *loc. cit.*

[2] Langley, *Journ. Physiol.*, Cambridge and London, 1880–2, vol. iii. p. 287; Boas, *Ztschr. f. klin. Med.*, Berlin, 1888, Bd. xiv. S. 249.

[3] *Loc. cit.*

[4] As tested by the inability of the filtrate to digest fibrin.

in twenty-four hours, but caused coagulation of fresh milk of neutral reaction in one to three minutes.

Such a solution of purified rennin behaves essentially differently in its reactions from a proteid solution. It is not coagulated by heat, does not give the xanthoproteic reaction, and is not precipitated by alcohol, tannin, iodine, or neutral acetate of lead; it is, however, precipitated by basic acetate of lead.

The Pancreatic Enzymes.

The pancreatic juice of all vertebrates in which it has been tested[1] contains three distinct enzymes, each of which acts on a different class of the three great divisions of foodstuffs.[2] In the invertebrates generally, the place of the pancreas is taken by the so-called liver, hepato-pancreas, or digestive gland. This usually contains enzymes, capable collectively of attacking all three classes of foodstuffs, and with varying reaction; so that this organ may be considered as taking the place of the combined digestive glands of the vertebrates.[3]

The different enzymes of the pancreas do not appear equally early in life; the pancreatic diastase, *amylopsin*, is not found at birth, but first appears a month or more afterwards.[4] The proteolytic ferment, *trypsin*, is found during the last third of fœtal life.[5] No similar information is on record regarding the fat-splitting ferment, *steapsin*.

The relative amounts of the different enzymes in pancreatic juice vary considerably. In passing from a flesh to a bread-and-milk diet, the proteolytic activity is said to diminish while the diastatic activity increases, and *vice versâ* in passing from a carbohydrate to a proteid diet.[6]

In addition to the methods of extraction already described under general methods, the pancreatic enzymes may be obtained in solution by various other methods, of which the following is a summary:—

1. By extracting with water saturated with chloroform; such an extract keeps well and is very efficient.[7]

2. By extracting with water containing 3 to 4 per cent. of a mixture of 2 parts of boracic acid and 1 part of borax.[8]

3. By placing the fresh gland, finely minced, in a saturated solution of sodium chloride. This gives a strong solution of the proteolytic and diastatic enzymes.[9]

4. By extracting the fresh pancreas, freed from fat and finely minced, with about four times its weight of 25 per cent. alcohol for four or five days; succeeded by filtration, which may be assisted by a trace of acetic acid.[10]

[1] For a detailed account of the action of pancreatic extracts in different animals, see Harris and Gow, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 469.

[2] A milk curdling enzyme is also present; see Milk, p. 127.

[3] Krukenberg, "Grundzüge einer vergleichenden Physiologie der Verdauung," Heidelberg, 1882.

[4] Korowin, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1873, Bd. iii; Zweifel, "Untersuch. ueber den Verdauungsapparat der Neugeborenen," Berlin, 1874.

[5] Albertoni, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1878, Bd. viii. S. 254.

[6] Vassiliew, *Arch. de sc. biol.*, St. Pétersbourg, 1893, vol. ii. p. 219; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1893, Bd. xxiii. S. 219.

[7] Roberts, "Lumleian Lectures," 1880; "Digestion and Diet," London, 1891, p. 18.

[8] Roberts, *loc. cit.*

[9] Roberts, *loc. cit.* Also recommended by Harris and Gow, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 469.

[10] Roberts, *loc. cit.*

5. A very active proteolytic extract may be obtained by extracting with water containing 0·01 to 0·05 per cent. of ammonia. The filtered extract gives a precipitate with acetic acid which digests proteid very energetically, and can be further purified.[1]

In preparing pancreatic extracts, it should be remembered that the gland does not at all periods contain the same amount of the ferments, or rather their zymogens, but that the amount fluctuates within wide limits according to the period after a meal. The pancreas of an animal in which digestion is not going on will yield little or no ferments; the best time is from four to seven hours after a meal. An inactive preparation may often be cured by making the extract faintly acid with acetic acid some time before using; this sets free ferment which may be present as zymogen in the extract.

Trypsin.[2]—The proteolytic enzyme of the pancreatic juice in the purest form in which Kühne obtained it, gave all the proteid reactions, thus differing from all the other purer forms of enzyme hitherto described. Kühne's product is decomposed on boiling, yielding 20 per cent. of albumin and 80 per cent. of peptone; it is soluble in water, but insoluble in anhydrous alcohol or glycerin. The insolubility of the purified dry product in anhydrous glycerin accords with v. Wittich's[3] observations, that both enzymes can be extracted from the *fresh* gland by glycerin; but if the gland mass be previously thoroughly dried by extraction with alcohol, glycerin only takes out the diastatic enzyme, the proteolytic one being left behind.

Influence of temperature.—The activity of trypsin increases, according to Roberts,[4] with rising temperature until 60° C. is reached, and then rapidly falls, all action ceasing between 75° C. and 80° C.; Biernacki[5] states that purified trypsin in 0·25 to 0·5 per cent. sodium carbonate solution is destroyed in five minutes by a temperature of 50° C., and in neutral solution by a temperature of 45° C. The presence of albumoses or of certain ammonium salts protects against the action of elevated temperature in alkaline solution.

Influence of reaction.—Kühne[6] made the observation that the activity of trypsin was permanently destroyed by digesting its solutions with pepsin and hydrochloric acid, and attributed the greater share in this action to the pepsin. Boas[7] afterwards showed that the destruction might be due to acid action alone, by demonstrating that addition of hydrochloric acid to the filtered intestinal contents causes a precipitate containing nearly all the ferments. This precipitate, on standing for a few hours under the acid, became inert, but, when quickly separated and redissolved in sodium carbonate, showed both diastatic and proteolytic action. The matter has recently been again tested by Melzer,[8] who

[1] Hammarsten, "Lehrbuch," Wiesbaden, 1895, Aufl. 3, S. 265.

[2] So named by Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1876, N. F., Bd. i. S. 190. Danilewski (*Virchow's Archiv*, 1862, Bd. xxv. S. 279) had previously to this obtained a product which failed to give many of the usual proteid reactions.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 198; Hüfner (*Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 360) failed to obtain a similar result, probably through using glycerin containing water.

[4] *Proc. Roy. Soc. London*, 1881, vol. xxxii. p. 158.

[5] *Ztschr. f. Biol.*, München, 1891, Bd. xxviii. S. 51.

[6] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1876, N. F., Bd. i. S. 193.

[7] *Ztschr. f. klin. Med.*, Berlin, 1890, Bd. xvii. S. 170.

[8] Inaug. Diss., Erlangen, 1894; Melzer's figures show that most of the destruction is due to acid alone.

finds that hydrochloric acid alone does destroy trypsin, but not so rapidly as when pepsin is also present.

All possible opinions have been held by various observers as to the reaction with which trypsin acts, and acts best;[1] it is now generally accepted that it can act either in an alkaline, neutral, or *very faintly* acid solution, but that the optimum reaction is that given by about 1 per cent. sodium carbonate (Na_2Co_3).[2]

Active proteolysis by trypsin cannot take place in presence of an acid reaction, except the acid be combined with proteid. If the proteid be completely saturated with acid, the rate is greatly slackened even when there is no free acid in the solution; and if much proteid be present, the ferment action may be abolished even before this stage is reached.[3]

Heidenhain[4] states that the concentration of sodium carbonate necessary to ensure maximum activity varies with the richness in ferment of the solution experimented upon; the richer in ferment, the lower the percentage of sodium carbonate necessary for maximum action.

Other alkaline carbonates are much less effective than sodium carbonate in increasing the activity of trypsin. The action is also said to be assisted, but to a still less degree, by other salts of the alkalies.[5]

Organic acids have not nearly so destructive an action as hydrochloric acids, arsenious acid has no hindering effect, and salicylic acid only when in saturated solution.[6]

The nature of the proteid submitted to digestion by trypsin has also a profound effect upon the rapidity of the process. Fresh unboiled fibrin is so quickly dissolved that it cannot be used as a comparative test for trypsin, and fibrin which has been boiled, or discs of hard-boiled white of egg, must be substituted for it.

Amylopsin.—An active amylolytic extract of pancreas can best be prepared by following Roberts' method of extracting with dilute alcohol.

Pancreatic juice is much more intensely diastatic than saliva, but it cannot be determined, until some method for isolating the diastases has been discovered, whether this is due to a difference in the amylolytic ferments present or to a mere difference in concentration. It is certain, however, that salivary, pancreatic, and malt diastases are practically identical in the qualitative character of their action on starch. Roberts

[1] See Corvisart, "Collection de mémoires sur une function peu connue du pancreas, la digestion des aliments azotés," Paris, 1857–8, p. 41; Meissner, *Ztschr. f. rat. Med.*, 1859, 3 Reihe, Bd. vii. S. 17; Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, N. F., Bd. i. S. 190; Danilewski, *Virchow's Archiv*, 1862, Bd. xxv. S. 291; May, *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1880, Bd. iii. S. 378; Lindberger, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1883, Bd. xiii. S. 280; Ewald, *Ztschr. f. klin. Med.*, Berlin, 1880, Bd. i. S. 615; Langley, *Journ. Physiol.*, Cambridge and London, 1880–2, vol. iii. p. 262.

[2] Weiss, *Virchow's Archiv*, 1876, Bd. lxviii. S. 413; Melzer, Inaug. Diss., Erlangen, 1894. According to the latter author, a digestion which is complete in two and a half hours with 1 per cent. Na_2CO_3 is incomplete in twenty-four hours with either 3 per cent. Na_2Co_3 or 0·010 per cent. of HCl.

[3] Chittenden and Cummins, *Stud. Lab. Physiol. Chem.*, New Haven, 1885, vol. i. p. 100.

[4] Hermann's "Handbuch," Bd. v. (1), S. 187.

[5] Podolinski, "Beitr. z. Kenntniss des pankreatische Eiweissfermentes," Breslau, 1876, S. 43. See also Chittenden and Cummins, *loc. cit.*, who found that borax and potassium cyanide augment, while salts of mercury and iron decrease, the activity.

[6] Lindberger, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1883, Bd. xiii. S. 280; Schäfer u. Bohm, *ibid.*, 1872, Bd. ii. S. 363; Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, N. F., Bd. i. S. 190.

states that pancreatic diastase is capable of converting 40,000 times its weight of starch into maltose and dextrin; Kröger, that 1 grm. of pancreatic juice, containing 0·021 grm. of dry solid, of which in turn only a small fraction could be amylopsin, digested in half an hour 4·67 grms. of starch.

Influence of temperature.—The rate of conversion increases with rising temperature from 0° C. to 30° C.; from 30° C. to 45° C. the rate is at a maximum and practically constant. Above 45° C. the action becomes slower with rising temperature, and ceases between 60° C. and 70° C., the ferment being here destroyed.[1]

Influence of reaction.—Pancreatic diastase closely resembles salivary diastase in its behaviour to change in reaction of the medium in which it is dissolved. It seems to act best when neutralised or in presence of *minute* traces of acid; but a limit of acidity is soon reached beyond which the rapidity of action rapidly diminishes, and the enzyme itself is quickly destroyed.[2] The optimum activity, according to Melzer's measurements, coincides with the presence of 0·01 per cent. of hydrochloric acid.

Pialyn.—Very little is known of the fat-splitting enzyme, pialyn, of the pancreatic juice. That the action is due to an enzyme is shown by the following experimental observations:—(*a*) The action is destroyed when the pancreatic juice or active pancreatic extracts are boiled; (*b*) it takes place in presence of antiseptics, and hence cannot be due to bacteria.[3]

The enzyme is much less stable than either of the other two associated with it in pancreatic juice. It is especially susceptible to the action of acids, being quickly destroyed by all except the higher fatty acids, so that great care to avoid acidity of solution must be exercised in the preparation of it from the pancreas. Paschutin[4] recommends for its extraction a dilute solution of sodium carbonate and bicarbonate in water, and Grützner[5] that it should be extracted from the perfectly fresh pancreas with a solution containing 90 c.c. of glycerin to 10 c.c. of 1 per cent. sodium carbonate, ten times the weight of gland to be extracted being taken of this fluid. However extracted, *it must be taken from a fresh gland and not from one which has stood over a day, as in the case of the other two enzymes*, for thereby an acid reaction would be developed, and as a consequence the fat-splitting enzyme would be destroyed.

The rapidity of action of the enzyme is at first increased by rising temperature. It acts almost twice as fast at 38° C. as at 18° C., but it is destroyed by boiling; the temperature of destruction is not accurately known.

It acts more slowly in the presence of 0·25 per cent. of sodium carbonate than in neutral solution.

Its activity is greatly increased by the presence of bile, still more by a mixture of bile and hydrochloric acid; this increase in activity is due to the bile salts or bile acids, which have a similar effect. The rapidity of action of the enzyme is usually much underrated, and it

[1] Roberts, "Digestion and Diet," London, 1891, p. 74; *Proc. Roy. Soc. London*, 1881, vol. xxxii. p. 145.

[2] Melzer, Inaug. Diss., Erlangen, 1894.

[3] Nencki, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xx. S. 367.

[4] *Arch. f. Anat. u. Physiol.*, Leipzig, 1873, S. 386.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 302.

is probable that it is capable of splitting up all the fat of a full meal in the ordinary time of digestion within the body.[1]

Pialyn acts on other esters than the neutral fats causing a similar saponification.[2]

Separation of the pancreatic enzymes.—There has, strictly speaking, been no complete isolation of the pancreatic enzymes obtained by the various workers on the subject. Partial success has been so far obtained, in that methods have been invented which yield solutions much richer in one of the two principal enzymes than in the other.

Danilewski[3] was the first to tackle this difficult task, under the direction of Kühne. He found that, after shaking up a watery infusion of the pancreas of the dog with excess of magnesia, and filtering, there remained an infusion which possessed only a proteolytic and diastatic action.

This solution was mixed up with one quarter of its volume of thick collodion solution (in alcohol and ether), and thoroughly shaken. The collodion is thrown out of solution as a pasty mass, which mechanically carries with it the proteolytic ferment, while the diastatic ferment remains in solution. The collodion is removed, washed, and dissolved in a mixture of alcohol and ether. This solution is allowed to stand for some days, when the proteolytic ferment with a little proteid falls to the bottom as a yellow sediment. This sediment, when dissolved again in water, digests fibrin in alkaline or neutral, not in acid solution. The filtrate from the collodion, which contains the diastatic enzyme, is evaporated down *in vacuo*, and filtered from anything which precipitates out. The filtrate is precipitated by excess of absolute alcohol, extracted by a mixture of 2 parts water to 1 part alcohol, and dried *in vacuo*. The solution so obtained rapidly converted starch into sugar, and only possessed a very feeble action on fibrin. Lossnitzer[4] has repeated these experiments, and only partially confirms them. Neither of the two products obtained by Danilewski gave the xanthoproteic, or Millon's reactions.

Cohnheim[5] obtained the diastatic enzyme from an infusion of pancreas, by a method identical with that by which he obtained ptyalin.[6] This substance possessed no proteolytic action, did not give the proteid reactions, but acted very energetically on starch.

v. Wittich[7] made use of the insolubility of trypsin in dry glycerin to obtain an extract rich in diastatic ferment and free from proteolytic action. The pancreas is dehydrated in strong alcohol, and further allowed to stand under absolute alcohol for some time; the tissue is then dried and extracted with dry glycerin; the extract after filtration is precipitated by excess of alcohol, and the precipitate is again extracted with dry glycerin. In this manner v. Wittich obtained an extract which did not act on fibrin and had an intense action on starch. Hüfner got an extract, on repeating the process, which also contained trypsin; but as Kühne states that trypsin is not soluble in glycerin, Hüfner's results may be due to water in the glycerin employed.

Paschutin[8] attempted to separate the pancreatic enzymes by using as

[1] All these observations on the rapidity of action of this enzyme, and its variations, have been made by Rachford, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 72.

[2] Berthelot, *Ann. d. chim.*, Paris, 1854, tome xli. p. 272; Nencki, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xx. S. 367; Baas, *Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xiv. S. 416.

[3] *Virchow's Archiv*, 1862, Bd. xxv. S. 279.

[4] *Arch. d. Heilk.*, Leipzig, 1864, Bd. v. S. 556.

[5] *Virchow's Archiv*, 1863, Bd. xxviii. S. 241.

[6] See p. 328.

[7] *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 198.

[8] *Arch. f. Anat. u. Physiol.*, Leipzig, 1873, S. 382.

extractives concentrated solutions of different salts. He found that some salt solutions extracted all three ferments, while others especially extracted one ferment accompanied by traces of the others. Thus sodium chloride, sodium sulphate, and potassium chlorate extracted all three ferments indifferently; sodium bicarbonate, with a little of the normal carbonate added, extracted best the fat-splitting ferment; the proteolytic ferment was taken up best by potassium iodide, arsenite, or sulphite; and the diastatic ferment by potassium arsenate alone or with the addition of ammonia.

Dastre[1] has recently described methods for approximately separating the proteolytic and diastatic enzymes of the pancreas.

1. If the pancreas of an animal killed during digestion be cut into large pieces, and these then digested for 15–20 minutes at 40° C., in normal saline (·7 per cent.), the filtrate is found to possess a strong diastatic action, but scarcely any proteolytic action. If, after this first extraction, the pieces are finely minced and extracted anew with normal saline (1 per cent. of sodium fluoride being added to prevent putrefaction), an extract is obtained rich in proteolytic ferment, but containing scarcely any diastase.

2. On extracting a fresh gland with alcohol of increasing strength, afterwards with ether, and drying over sulphuric acid, a powder is obtained which yields, on extraction with saline, a fluid which is almost inert towards starch, but is actively proteolytic.

3. An extract made from the pancreas of an animal which has not been fed for some days, contains proteolytic ferment but has scarcely any diastatic action.

The Intestinal Enzymes.

Practically nothing is known of the enzymes of the small intestine save their action on foodstuffs; none of them have been obtained in even approximately pure condition, and the fact that there are enzymes rests on the observations—(1) that the action is destroyed by boiling, and (2) that it takes place under antiseptic conditions. Until the importance of this latter condition was demonstrated by the work of Kühne on pancreatic digestion, there was much difference of opinion as to whether the succus entericus contained a proteolytic enzyme or not; some observers had observed digestion of proteids by this fluid, and others had been unable to do so. At length it was shown by Masloff[2] and by Wenz[3] that when precautions are taken to prevent bacterial growth, the succus entericus or extracts of the intestinal mucous membranes have no action on proteids or on albumoses.

With regard to the action of succus entericus on carbohydrates, the more recent work on the subject all goes to show that starch is converted into maltose, maltose into dextrose, and cane-sugar into dextrose and lævulose, both by the succus entericus and by extracts of the intestinal mucous membrane.

The succus entericus contains no enzyme which acts on neutral fats. The power of emulsifying fats, which was occasionally observed by the earlier workers on the subject, was doubtless due to the alkalinity of the

[1] *Compt. rend. Soc. de biol.*, Paris, 1893, tome xlv. p. 648; *Arch. de physiol. norm. et path.*, Paris, 1893, tome xxv. p. 774.

[2] *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1882, Bd. ii. S. 920. Masloff found *very slight* action of the juice *when acidified*, probably due to infiltrated pepsin.

[3] *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 1. This result is confirmed by the observations of Tubby and Manning on human succus entericus, *Guy's Hosp. Rep.*, London, 1891, vol. xlviii. p. 277.

fluid, aided by the presence of free fatty acid in the fat used for the experiments.[1]

Paschutin[2] attempted by two different methods to separate the diastatic and inverting ferments:—

1. An infusion of the intestinal mucous membrane was made by rubbing it up with water and powdered glass, and filtering. When this infusion was mixed with a solution of collodion, the precipitated collodion brought down most of the inverting enzyme, and most of the diastatic enzyme was left in solution, but only a partial separation could be effected in this manner.

2. The mucous coat of a piece of intestine was freed from the other coats, and then water was filtered through this, under pressure. The fluid which filtered through acted energetically on starch, but had no action or only a very feeble one on cane-sugar.

THE CHEMICAL COMPOSITION OF THE DIGESTIVE SECRETIONS.

Saliva.

The saliva is a mixture in varying proportions of the secretions of the different salivary glands. As these secretions differ from one another considerably in chemical composition, it will be well to consider first the physical and chemical nature of each of them in turn, and afterwards that of the fluid which results from their admixture.

Submaxillary saliva.—Submaxillary saliva may be obtained by inserting a fine cannula into the opening of Wharton's duct. In some individuals Wharton's duct carries to the mouth the secretion of the submaxillary gland only, in others the duct of Bartholin leads into Wharton's duct, when the latter conducts the mixed secretion of the submaxillary and sublingual glands to the mouth. The tongue should be raised, but not too high, the cannula carefully inserted and gently pushed into the duct for about an inch. By this procedure the end of the cannula is thrust past the opening of the duct of the sublingual gland, in case both glands share a common duct, and so the obtaining of submaxillary saliva only is ensured.[3]

Human submaxillary saliva is a clear, watery, mobile fluid, which becomes viscid on standing in contact with air, and deposits flocculi. It is always alkaline in reaction. On boiling, it becomes cloudy, and the cloudiness is increased by the addition of acid. Its specific gravity varies between 1·0026 and 1·0033, and is lessened by hunger. The amount of total solids lies between 0·36 and 0·46 per cent., and is not much influenced by food. According to Eckhard, it contains no sulphocyanates, while Oehl and Sertoli[4] state that it contains them, but in less amount than the secretion of the parotid. Colorimetric

[1] See, however, Schiff, *Arch. de physiol. norm. et path.*, Paris, 1892, tome xxiv. p. 679. Schiff here repeats his earlier statements, that succus entericus acts both on proteids and neutral fats. Prege (*Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxi. S. 359) has recently obtained succus entericus from a Vella fistula in the sheep, and determined that it has no action on proteids or neutral fats.

[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1871, S. 305.

[3] Eckhard, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1862, Bd. i. S. 126; cited from Maly, Hermann's "Handbuch," Bd. v. (2), S. 17.

[4] Oehl, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1865, Bd. i. S. 120; Sertoli, *ibid.*, 124.

measurements gave for the submaxillary saliva 0·004 per cent., for the parotid 0·03 per cent., of this substance, reckoned as potassium sulphocyanate. It contains ptyalin, as shown by its powerful diastatic action on starch.

The submaxillary saliva in the dog contains much more mucin than in man, and is in consequence much more viscid. It is alkaline in reaction, 100 grms. requiring for neutralisation 0·135 to 0·144 grms. of sulphuric acid, reckoned as SO_3. On standing in contact with air, calcium carbonate is thrown down as a flocculent precipitate, which was previously held in solution by the dissolved carbon-dioxide as bicarbonate. The same result is brought about more rapidly by heating. It contains, at most, only traces of proteid or of sulphocyanate. Its specific gravity is 1·0026 to 1·004.

The quantitative composition of the saliva obtained on stimulation of the submaxillary gland varies according to the nerve stimulated. The saliva obtained on stimulation of the sympathetic (sympathetic saliva) is scanty in quantity, and contains much mucin, which gives it a viscid consistency. Chorda saliva, on the other hand, is plentiful in quantity, contains less mucin, and is hence a thin watery fluid. The chorda saliva has a specific gravity of 1·0049 to 1·0056, and contains 1·2 to 1·4 per cent. of total solids; sympathetic saliva has a specific gravity of 1·0075 to 1·018, and contains 1·6 to 2·8 per cent. of total solids.[1]

Parotid saliva.—Human parotid saliva may be obtained by introducing a fine cannula into Stenson's duct.[2]

It is a thin, mobile fluid, usually clear, sometimes somewhat turbid, and contains no formed element save epithelial cells. It is alkaline in reaction, but the first few drops secreted may be neutral or acid, especially in a state of hunger; in all cases the alkalinity is less than that of submaxillary saliva.[3] Its specific gravity seems to be very variable (Mitscherlich, 1·006 to 1·008; Oehl, 1·010 to 1·012 with scanty secretion, 1·0035 to 1·0039 with plentiful secretion; Hoppe-Seyler, 1·0061 to 1·0088); the amount of total solids lies between 5 and 16 parts per thousand. It contains traces of proteids, but is free from mucin; it also contains ptyalin and sulphocyanate.

The parotid saliva of some animals, such as the dog and horse, is very rich in calcium bicarbonate, and often deposits crystals of calcium carbonate on standing.[4] Stimulation of Jacobson's nerve in the dog produces a flow of saliva from the parotid, poor in organic constituents. If, before this is done, the cervical sympathetic be stimulated, which alone produces no effect, on now stimulating the nerve of Jacobson a flow of saliva is obtained which is much richer in organic constituents.[5]

Sublingual saliva.—Oehl attempted to obtain human sublingual saliva by a similar method to that described in the case of the other two glands; he was only able to obtain a very small quantity, insufficient for

[1] Eckhard, *Beitr. z. Anat. u. Physiol.* (Eckhard), Giessen, 1860, Bd. ii. For further details regarding the influence of nerves on the composition of saliva, see article on "Mechanism of Salivary Secretion."

[2] Eckhard, *loc. cit.*; Oehl, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1865, Bd. i. S. 120. See also Brunton in Sanderson's "Handbook of the Physiol. Laboratory," p. 467.

[3] See Astaschewsky, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1878, Bd. viii. S. 234; Fubini, *ibid.*, S. 235.

[4] Lehmann, "Physiol. Chem.," Bd. ii. S. 13.

[5] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 28; also in Hermann's "Handbuch," Bd. v. (1), S. 55.

quantitative analysis, but made out that it was a clear slimy fluid of stronger alkaline reaction than submaxillary saliva, and containing mucin, diastatic ferment, and sulphocyanide.

The sublingual saliva of the dog is a viscous, scarcely fluid mass; it contains salivary corpuscles, but is otherwise quite clear and transparent, is alkaline in reaction, and contains 2·75 per cent. of total solids.[1] Werther[2] has analysed the sublingual saliva of the dog, and finds that its great viscidity is not due to any excess of organic constituents; he attributes it to the reaction which he found to be neutral or barely alkaline. The proportion of inorganic salts is much larger than in parotid or submaxillary saliva.

Secretion of the mucous glands of the mouth.—This has not been obtained in man. In the dog it has been obtained by ligaturing the ducts of all the salivary glands, or by extirpating the salivary glands. The amount secreted is exceedingly small; it is a thick ropy mucus of alkaline reaction, full of fragments of epithelial and mucous cells, and containing about 1 per cent. of total solids.[3]

The mixed saliva.—Mixed saliva may easily be obtained from the mouth by depressing the head and everting the lower lip; or by depressing the head, keeping the mouth widely open, and avoiding all attempts to swallow. It is a clear, viscid, and very slightly opalescent fluid, which froths easily. It is normally alkaline in reaction; when it is acid this reaction is commonly due to fermentation of particles of food in the mouth. The alkalinity is least when fasting, as in the morning before breakfast, and reaches its maximum with the height of secretion during, or immediately after eating. According to Chittenden and Ely,[4] the alkalinity is equivalent to that of a solution containing 0·08 per cent. of sodium carbonate (Na_2Co_3).

The quantitative composition of mixed saliva is very variable, as might be expected from the difference in composition of the secretions which form it, and the varying proportion in which these must be present in different samples. The amount of total solids in human saliva varies normally between 5 and 10 parts per 1000; the specific gravity between 1·002 and 1·008.

Organic constituents.—The organic matter is partially in suspension, and partially in solution. The suspended matter consists of squamous cells detached from the epithelium of the mouth, and of the *salivary corpuscles*, which are leucocytes altered by the action of the saliva, and containing granules which exhibit in fresh saliva active Brownian movements. The dissolved organic matter consists of mucin, ptyalin, and traces of proteids; the amount of the latter is so small that it cannot be quantitatively estimated. Saliva is also said to contain normally minute traces of urea; but the amount is so small that such a statement cannot be made with certainty. In pathological conditions the amount of urea present may, however, become very appreciable. Leucine and lactic acid are found under

[1] Heidenhain, *Stud. d. physiol. Inst. zu Breslau*, Leipzig, Heft 4.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 293. See also Langley and Fletcher, *Phil. Trans.*, London, 1887, vol. clxxx. p. 109.

[3] Bidder and Schmidt, "Die Verdauungssäfte," Mitau und Leipzig, 1852, S. 5.

[4] *Am. Chem. Journ.*, Baltimore, 1883, p. 329. See also Werther, *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 293.

pathological conditions, but are not normal constituents. Grape-sugar and bile pigments are never found even in the severest cases of diabetes or icterus.[1]

Inorganic constituents.—These consist of salts of the alkalies (chiefly sodium) and alkaline earths; principally as chlorides, but also as phosphates and carbonates.[2] Calcium carbonate often separates from saliva, as a thin surface film, or as a cloudiness in the fluid on standing; this is due to the escape of carbon-dioxide by which the calcium was held in solution as bicarbonate. A precipitation of the calcium of the saliva, partially as carbonate and partially as phosphate, in the ducts of the salivary glands, often gives rise to *salivary concretions*; and a similar deposit, mixed with phosphate and traces of silica, forms the tartar of teeth.

Sulphocyanate of saliva.—Treviranus,[3] as early as 1814, observed that when a dilute solution of ferric chloride is added to saliva a reddish coloration is obtained. This was even before sulphocyanic acid was known chemically, and Tiedemann and Gmelin[4] afterwards proved that the effect was due to the presence of a sulphocyanate.

The amount of sulphocyanate is not large. Oehl states it as equivalent to 0·00016–0·0084 per cent., estimated as potassium sulphocyanate, and Munk as equivalent to 0·01 per cent. sulphocyanic acid, or 0·014 per cent. of sodium sulphocyanate.[5] The presence of a sulphocyanate in saliva may be demonstrated qualitatively in several ways—(1) A very greatly diluted and slightly acidulated solution of ferric chloride is added drop by drop to saliva, when a reddish coloration is obtained, if sulphocyanate is present in normal quantity; the colour disappears on adding mercuric chloride. This test is difficult to obtain. (2) A filter paper is dipped in a *weak* solution of ferric chloride, containing a trace of hydrochloric acid, and then allowed to dry, when it should have only a faint amber colour. On such test paper a drop of saliva produces a reddish stain.[6] (3) Filter paper is impregnated with tincture of guaiacum, and then drawn through a solution of 0·05 per cent. copper sulphate. On such paper, saliva containing sulphocyanate causes a blue stain.[7] (4) Saliva is treated with iodic acid, when iodine is set free; this in turn is treated with starch paste, when the blue compound of starch and iodine appears. This is said to be an exceedingly delicate reaction, showing a most minute trace of sulphocyanate, and not being produced by saliva free from sulphocyanate,[8] but iodic acid is an exceedingly unstable compound, and the latter statement is questionable.

Sulphocyanate is often absent in human saliva. Some authors state that it is found in dog's saliva, others that it is not; the explanation may be that its presence in dog's saliva is also not constant. It is said to be absent in the saliva of the horse, ox, sheep, goat, and pig.[9] Leared[10] found that sulpho-

[1] See Maly, Hermann's "Handbuch," Bd. v. (2), S. 8.

[2] The older analysts were accustomed to proportion the bases and acids, on the supposition that as much as possible of the so-called strongest acid was combined with the strongest base. We now know that the acids and bases are distributed according to definite laws, and no longer speak of so much chloride, for example, as existing in a complex mixture, but state separately so much sodium and so much chlorine, etc.

[3] "Biologie," 1814, Bd. iv. S. 330.

[4] "Die Verdauung nach Versuchen," Bd. i. S. 8.

[5] See Maly, Hermann's "Handbuch," Bd. v. (2), S. 10, 14.

[6] Gscheidlen, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, Bd. iv. S. 91.

[7] Böttger, *ibid.*, 1872, Bd. ii. S. 204.

[8] Solera, *ibid.*, 1877, Bd. vii. S. 256; 1878, Bd. viii. S. 235.

[9] Ellenberger and Hofmeister, "Vergleich. Phys. d. Haustiere," Berlin, 1890, S. 495.

[10] *Proc. Roy. Soc. London*, 1870, vol. xviii. p. 16.

cyanate was present in the blood and urine as well as in the saliva. Gscheidlen [1] and Munk [2] have also found it in urine.

An exalted importance has been given to the sulphocyanate in saliva, from its supposed origin from proteid, and from its assumed value as an indicator of the rate of proteid metabolism. Sulphocyanic acid has a similar constitution to cyanic acid, an atom of sulphur merely replacing an atom of oxygen, and the ammonium salt of sulphocyanic acid undergoes a similar decomposition to that of cyanic acid, yielding sulpho-urea instead of urea, thus :—

Oxygen compounds—	$CN.OH$; (cyanic acid)	$CN.O.NH_4$; (ammonium cyanate)	$CO.(NH_2)_2$ (urea)
Sulphur compounds—	$CN.SH$; (sulphocyanic acid)	$CN.S.NH_4$; (ammonium sulphocyanate)	$CS.(NH_2)_2$ (sulpho-urea)

From this relationship, from the presence of sulphur in its molecule, and from its presence in the urine, and in traces in the blood, it is probable that the sulphocyanate of the saliva is a product of proteid metabolism. Fenwick [3] has investigated the variation in the sulphocyanate of the saliva, especially in relation to the variations in the nutrition of the body under pathological conditions. He states that the amount of sulphocyanate bears a relationship to the amount of sulphur (as taurocholates) in the bile, and that when the bile is diverted from the alimentary canal the sulphocyanate of the saliva disappears. That it would be dangerous to take the amount of sulphocyanate as any gauge of the amount of proteid metabolism, is shown by its complete absence in many species of animals, and in many individuals where it is normally present in a species ; this does not make any the less probable the statement that in those individuals in which sulphocyanate is present its quantity should vary with the activity of proteid metabolism.

Saliva also contains traces of nitrites,[4] which may be demonstrated by diluting saliva with five times its volume of water, making acid with sulphuric acid and adding a solution of metadiamido-benzol, when an intense yellow colour is produced. In this way Griess estimated colorimetrically the amount of nitrite in saliva at 1–10 mgrms. per litre. Minute traces of ammonia may also be shown to be present in saliva by the addition of Nessler's reagent.[5]

Gases of the saliva.—The saliva holds considerable volumes of gas in solution or in chemical combination. In human parotid saliva, Külz [6] found in 100 vols. of saliva, of oxygen, 1·46 vols. ; of nitrogen, 2·8 vols. ; and of carbon-dioxide 66·7 vols., of which latter 62 vols. were in chemical combination. In submaxillary saliva of the dog, obtained by stimulating the chorda tympani, Pflüger [7] found 0·5–0·8 vols. of oxygen, 0·9–1·0 vols. of nitrogen, and 64·73–85·13 vols. of carbon-dioxide, most of the latter being chemically combined. These figures are interesting, both because of the large amount of carbon-dioxide present, and the fact that the oxygen exceeds the amount dissolved by blood plasma.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 401.
[2] *Virchow's Archiv*, 1877, Bd. lxix. S. 354.
[3] Fenwick, "The Saliva as a Test for Functional Disorders of the Liver," London, 1889.
[4] Schönbein, *Journ. f. prakt. Chem.*, Leipzig, Bd. lxxxvi. S. 151 ; Schaer, *Ztschr. f. Biol.*, München, Bd. vi. S. 467 ; Griess, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, Bd. ii. S. 624.
[5] Maly, Hermann's "Handbuch," Bd. v. (2), S. 8. See also W. Sticker, *München. med. Wchnschr.*, 1896, Bd. xliii. Nos. 42–43.
[6] *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 321.
[7] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 686.

Tables of Analyses of Saliva.

TABLE I.

SUBMAXILLARY SALIVA.

	I.	II.	III.	IV.	ASH OF No. III.			GASES. I.	GASES. II.
Water . .	991·45	996·04	994·4	991·14	K_2SO_4	0·209	in 1000 parts of saliva.	CO_2 free 19·3	22·5
Total Solids .	8·55	3·96	5·6	8·86	KCl	0·940		CO_2 combined 29·9	42·5
(*a*) Organic .	2·89	1·51	1·75	3·53	Na Cl	1·546		Nitrogen 0·7	0·8
(*b*) Inorganic	5·66	2·45	3·85	5·33	Na_2CO_3	0·902		...	...
1. Soluble	4·50	...	3·59	5·27	$CaCO_3$	0·150		Oxygen 0·4	0·6
2. Insoluble	1·16	...	0·26	0·06	$Ca_3(PO_4)_2$	0·113			

Analyses I. and II. are of dog's saliva, by Bidder and Schmidt.[1] Analysis III. is by Herter.[2] Analysis IV. is of cow's saliva.[3] The ash analysis is by Herter.[4] The gas analyses are of dog's saliva by Pflüger.[5]

TABLE II.

PAROTID SALIVA.

	I.	II.	III.	IV.	V.	VI.	VII.
Water . .	985·4–983·7	993·16	995·3	991·5–993·8	990·00	990·7	869·0
Total Solids .	14·6–16·3	6·84	4·7	8·47–6·1	10·0	9·3	11·0
(*a*) Organic .	9·0	3·44	1·4	1·53	2·06–6·0	0·44	1·0
(*b*) Inorganic .	5·3	3·40	3·3	6·93	4·8–8·73	8·82	10·0
1. Soluble .	...	...	2·1	6·25	...	8·72	10·0
2. Insoluble	...	...	1·2	0·68	...	0·10	Traces

This table has been compiled from Maly.[6] I. and II. are analyses of human parotid saliva, by Mitscherlich and Hoppe-Seyler respectively. The former states the amount of the sulphocyanate in his sample at 0·3 per thousand. III. and IV. are of dog's parotid saliva, by Jacubowitsch and Herter respectively; that given as soluble is set down by them as $CaCO_3$. V. is of horse's parotid saliva by Lehmann. VI. and VII. of the cow's and ram's parotid saliva respectively, by Lassaigne.

TABLE III.

SUBMAXILLARY, PAROTID, AND SUBLINGUAL SALIVA.

	Submaxillary Saliva.				Parotid Saliva.			Sublingual Saliva.			
	I.	II.	III.	IV.	I.	II.	III.	I.	II.	III.	IV.
Water . . .	987·7	988·7	983·2	987·4	991·4	992·6	992·6	978·8	984·7	986·3	957·2
Total Solids .	12·3	11·3	16·8	12·5	8·6	7·4	8·1	21·2	15·3	13·7	12·8
(*a*) Organic .	...	6·6	10·2	6·2	...	0·6	4·0	...	1·9	4·3	3·4
(*b*) Inorganic .	...	4·7	6·6	6·4	...	6·8	4·1	...	13·4	9·4	9·4
1. Soluble .	5·6	4·3	5·8	6·0	5·6	6·4	3·6	11·0	12·7	9·0	9·3
2. Insoluble .	...	0·42	0·73	0·42	...	0·45	0·54	...	0·68	0·44	0·17
Alkalinity— (Na_2CO_3) .	1·6	1·7	1·1	...	1·9	1·7	1·7	...	...	...	...
Chlorides— (as NaCl) .	3·35	1·5	3·29	...	2·39	0·78	0·85	7·06	10·8	8·14	...

[1] Maly, Hermann's "Handbuch," Bd. v. (2), S. 19.
[2] Hoppe-Seyler, "Physiol. Chem.," Bd. ii. S. 191.
[3] Lassaigne, cited by Maly, *loc. cit.* [4] *Loc. cit.* [5] Maly, *loc. cit.*
[6] Hermann's "Handbuch," Bd. v. (2), S. 16, 17; Hoppe-Seyler, "Physiol. Chem.," Bd. ii. S. 198, 199.

This table shows the results obtained by Werther [1] in four experiments on dogs, in which all three kinds of saliva were collected and analysed. The results have been placed in a similar form to that of the other tables, for ease of comparison. It should be observed that the sublingual saliva was barely alkaline in all four experiments, while the submaxillary saliva was only so in one experiment; that the sublingual saliva contains in spite of its viscidity no more organic matter than the others, while it does contain much more chlorides. Human sublingual saliva has never been obtained in sufficient quantity for analysis.

TABLE IV.

Buccal Mucus.

Water	990·02
Total solids	9·98
Organic matter—	
(*a*) Soluble in alcohol	1·67
(*b*) Insoluble in alcohol	2·18
Inorganic salts—	
Chiefly chloride and phosphate of sodium	6·13

From Bidder and Schmidt, quoted by Maly.[2]

TABLE V.

Mixed Saliva.

	I.	II.	III.	IV.	V.	VI.	VII.
Water	992·9	995·1	994·1	988·3	994·7	994·2	989·6
Total solids	7·1	4·84	5·9	11·7	5·3	5·8	10·3
Suspended solids (epithelium, mucus, etc.)	1·4	1·62	2·13	...	...	2·2	...
Soluble organic matter	3·8	1·34	1·42	...	3·27	1·4	3·58
Potassium sulphocyanide	...	0·06	0·10	...	...	0·04	...
Inorganic salts	1·9	1·82	2·19	...	1·03	2·2	6·79

Ash of Mixed Saliva.

	Human.	Dog.
Total solids (in 1000 parts of saliva)	1·82	6·79
Phosphoric acid	0·51	0·82
Soda	0·43	
Lime	0·03	0·15
Magnesia	0·01	
Alkaline chlorides	0·84	5·82

Analyses I. to VI. are of human saliva by Berzelius, Jacubowitsch, Frerichs, Tiedemann and Gmelin, Herter, and Hammerbacher respectively. Analysis VII. is of dog's saliva by Schmidt. The table is taken from Maly,[3] except Analysis VI., which is from Hammerbacher.[4] The analyses of ash are by Jacubowitsch.[5] In 1000 parts of the ash of mixed human saliva, Hammerbacher[6] found 457·2 of K_2O, 95·9 of Na_2O, 50·11 of Fe_2O_3, 1·55 of MgO, 63·8 of SO_3, 188·48 of P_2O_5, and 183·5 of chlorine.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 293.
[2] Hermann's "Handbuch," Bd. v. (2), S. 20.
[3] *Ibid.* Bd. v. (2), S. 14.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. v.
[5] Maly, *loc. cit.*
[6] *Loc. cit.*

Gastric Juice.

Human gastric juice mixed with water or food may be obtained for clinical purposes by the use of a gastric sound or the stomach pump, but pure gastric juice cannot be obtained in this way, because when the stomach is empty the secretion of gastric juice stops, and can only be initiated by the drinking of water or the taking of food.[1]

Notwithstanding the considerable number of cases of gastric fistula in man already enumerated, the details as to the quantitative chemical composition and physical characteristics of that fluid are very meagre. Only one set of complete analyses of the fluid has been carried out by Schmidt, and these, along with certain incomplete analyses by other observers of the total solids and amount of acid, are all the quantitative data we possess.

In a case of human gastric fistula, observed by C. Schmidt,[2] the fluid obtained was clear as water, less acid than dog's gastric juice, and had a specific gravity of 1·0022–1·0024. It scarcely became clouded on heating, and left on evaporation a brownish-yellow deliquescent acid residue, which on incinerating left a colourless, neutral, or faintly alkaline ash, containing no carbonates. On distilling the liquid, only water came over, until the fluid attained the consistency of oil, then traces of hydrochloric acid, which became stronger as the process was continued.

In a case observed by Richet, in which the œsophagus had been occluded by strong alkali, and the gastric fistula was the result of an operation, the gastric juice was also colourless, had a faint smell, and varied greatly in acidity.

Pure gastric juice has also recently been obtained by Fremont[3] from a fistula in the isolated stomach of the dog. Gastric juice so obtained is a limpid, clear, colourless, inodorous, very acid, and powerfully peptic fluid, capable of digesting its own weight of coagulated albumin. The dog in question weighed 12 kilos., and yielded 800 grms. of gastric juice daily. If the secretion takes place at the same rate in the human subject, a man weighing 60 kilos. (132 lbs.) should secrete 4 litres of gastric juice daily.

Pure gastric juice may be collected from a Pawlow fistula[4] twelve to fifteen hours after a true meal, by giving the animal a fictitious meal. The food which is eaten does not reach the stomach, but drops from an œsophageal fistula. The process of feeding induces reflexly an abundant secretion of gastric juice, which can be collected in a pure condition. A dog will go on feeding voraciously in this manner for hours, and in the course of an hour 200–300 c.c. of gastric juice may be collected. The animal is said to be unaffected in health by a collection of an hour per diem.

[1] This method is of more service clinically than physiologically as a mode of obtaining gastric juice in cases of dyspepsia, in order to determine the amount of acidity, and whether this is due to a normal amount of hydrochloric acid or to excess of organic acids, the product of bacterial action. See Leube, *Sitzungsb. d. phys.-med. Soc. zu Erlangen*, 1871, Heft 3; Külz, *Deutsche Ztschr. f. prakt. Med.*, Leipzig, 1875, No. 27; C. A. Ewald, "Klinik der Verdauungskrankheiten," 1890, Bd. i. S. 87; Gamgee, "Physiological Chemistry," vol. ii. pp. 163–178.

[2] The case was that of a healthy woman with a chronic fistula, yielding gastric juice freely without apparent effect on the health of the patient.

[3] Demonstrated by Herzen, International Congress, Bern, 1895.

[4] Pawlow and Schoumow-Simanowsky, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1889, Bd. iii. S. 113. See article on "Mechanism of Gastric Secretion."

Konowaloff [1] collected over 10 litres, as above described, and subjected the fluid to chemical examination. It was a clear, colourless, odourless fluid, which could be kept indefinitely without undergoing decomposition. When diluted with its own volume of water, it becomes somewhat cloudy; with four volumes of water, a permanent opalescence resulted, which on further dilution eventually disappeared. On neutralising with alkali, a flocky precipitate appeared, redissolving in the slightest excess. Cooling the juice to 10–11° C. caused a finely granular precipitate to appear, which dissolved again on warming. Its specific gravity averaged 1·00478; total solids, 0·478 per cent.; acidity, equivalent to 0·544 per cent. of hydrochloric acid. When the acid gastric juice is so removed the reaction of the urine becomes alkaline [2] (0·96–1·31 per cent. of Na_2O).

Freshly secreted gastric juice is said to contain traces of proteid,[3] which, on standing, is converted into albumoses and peptones; these, with traces of mucin, and the two enzymes, pepsin and rennin, are the only organic constituents.

The inorganic salts consist chiefly of chlorides (with traces of phosphates) of sodium, potassium, and calcium, and traces of magnesium and iron.

The total amount of solids in gastric juice is very small, seldom amounting to more than 2 per cent., and often being much less. Excess of alcohol causes a flocky precipitate containing all the organic matter.

Alkalies and alkaline carbonates added to gastric juice cause a cloudiness or a flocky precipitate of tricalcic phosphate, with traces of phosphates of iron and magnesium, and some organic matter. The precipitation of tricalcic phosphate by ammonia shows that calcium is present as acid phosphate in gastric juice.

Quantitative Composition of Gastric Juice.

	I.	II.	III.	IV.
	Human.	Dog.	Dog.	Sheep.
Water	994·40	973·06	971·17	986·14
Total solids	5·60	26·94	28·83	13·86
Organic matter	3·19	17·13	17·34	4·05
HCl	0·20 ?	3·34	2·34	1·23
$CaCl_2$	0·06	0·26	1·66	0·11
NaCl	1·46	2·50	3·15	4·37
KCl	0·55	1·12	1·07	1·52
NH_4Cl	...	0·47	0·54	0·47
$Ca_3(PO_4)_2$	0·125	1·73	2·29	1·18
$Mg_3(PO_4)_2$		0·23	0·32	0·57
$FePO_4$		0·08	0·12	0·33

The analyses are by C. Schmidt, quoted from Maly, Hermann's "Handbuch," Bd. v. (2) S. 70; *Ann. d. Chem.*, Leipzig, 1854, Bd. xcii. S. 42; and "Verdauungssäfte," S. 44.

Analysis I. is of human gastric juice, obtained from Schmidt's case of gastric fistula already quoted; it is evident that this gastric juice contained

[1] Inaug. Diss., St. Petersburg, 1893; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1893, Bd. xxiii. S. 289.

[2] Schoumow-Simanowsky, *Arch. de sc. biol.*, St. Pétersbourg, 1893, vol. ii. p. 462.

[3] Hammarsten, "Lehrbuch der physiol. Chem.," Wiesbaden, 1895, Aufl. 3, S. 233.

much saliva, as the total solids and amount of acid are much less than those usually found. Analyses II. and III. are of dog's saliva. Analysis II. gives the mean of ten determinations, in the case of a dog in which all the salivary ducts had been ligatured. Analysis III. gives the mean of three, in the case of a dog with normal salivary glands. Analysis IV. is that of the gastric juice of a sheep.

The acid of the gastric juice.—The acid of the gastric juice has probably given rise to more discussion than any other subject in physiological chemistry. The principal points for consideration are—(*a*) the nature of the acid; (*b*) the seat of formation and the mode of origin of the acid; (*c*) the function of the acid.

The nature of the acid.—Before discussing this question in detail, it may be well to state clearly the present state of opinion on the subject.

It has been demonstrated that hydrochloric acid is the principal acid of the gastric juice, and that in the purer samples free from food it is always present, and is almost exclusively *the only acid present*; while in gastric juice mixed with food, especially with carbohydrate food, it may be, and often undoubtedly is, accompanied by lactic acid. C. Schmidt, from a large number of painstaking and laborious analyses, concluded that the pure gastric juice of carnivora, obtained after a fast of eighteen to twenty hours, contains *only hydrochloric acid*, and no trace of lactic or acetic acids; while the gastric juice of herbivora contains, besides hydrochloric acid, small quantities of lactic acid, but this is even then probably from remnants of carbohydrate food.[1]

Prout,[2] in 1824, first showed that gastric juice contains free hydrochloric acid by the following method:—

The contents of a stomach were mixed up with water, and, after the mixture had settled, the clear part was removed by decantation. This was divided into three *equal* portions, *a*, *b*, and *c*.

(*a*) The first portion was evaporated to dryness, incinerated, and the total amount of chlorine in the ash determined by weighing, as silver chloride.

(*b*) The second portion was first made alkaline by the addition of potash, then evaporated to dryness, incinerated, and the total chlorine determined as before.

(*c*) In the third portion, the total acidity was determined by titration against standard alkali, and reckoned as hydrochloric acid.

In portion (*a*) all the free acid is driven off as well as any which may be combined with volatile or decomposable bases (such as ammonium chloride); in portion (*b*) all the chlorine remains, that which was either free or combined with ammonia becoming converted into non-volatile potassium chloride; therefore the difference of (*b*) and (*a*) gives the free hydrochloric acid, plus any volatile chlorides which may be present. In (*c*) all the acid is estimated as hydrochloric acid, and by subtracting this from the difference of (*b*) and (*a*) the amount present as volatile chlorides is obtained.

Prout also showed that when gastric juice is distilled, towards the

[1] The stomach of the herbivora retains food for a much longer period than that of carnivora. Traces of food are usually found in the stomach of the sheep even thirty-six hours after a meal. See Cl. Bernard, "Leçons de physiol. expér." 1856, tome ii. p. 389.

[2] *Phil. Trans.*, London, 1824, part i. p. 45.

end of the process hydrochloric acid passes over. In addition, he tried to obtain lactic acid from gastric juice, but with negative results.

The remarkable results so obtained by Prout were confirmed by Children[1] in England, by Braconnot[2] in France, and by Dunglison and Emmet,[3] with gastric juice obtained from Beaumont's case of fistula.

When the period at which they were carried out is considered, it must be admitted that these experiments of Prout were most ingenious, and he well deserves the honour of being the first to awaken the minds of men to the conception that the animal organism was capable of producing such a substance as hydrochloric acid.[4] Physiological chemists, however, were chary in believing that the gentler forces of the animal organism were capable of producing such a substance as hydrochloric acid, which they were unable to obtain experimentally except by the use of potent inorganic reagents. Accordingly, objections flowed in against Prout's work.

Claude Bernard and Barreswil[5] showed that when sodium chloride was added to a solution of lactic acid, and the mixture distilled, hydrochloric acid appeared in the distillate towards the end of the process when the mixture was beginning to grow solid. They concluded that the free acid of the gastric juice was lactic acid. Lehmann[6] ascribed the free hydrochloric acid of Prout's distillation experiment to the action of the lactic acid, concentrated by evaporation, on the calcium chloride also present in gastric juice. Many other observers were also agreed that the free acid in gastric juice was lactic acid.[7] Blondlot[8] about this time enunciated the hypothesis that the acidity of the gastric juice was due in part to acid calcium phosphate, and evolved a theory, closely resembling a much more recent one by Maly, as to the origin of the acid by the formation of this substance, accompanied by traces of hydrochloric and phosphoric acids in the stomach wall, from the sodium chloride and calcium phosphate of the blood.[9] In presence of hydrochloric acid it is now known that part of any calcium phosphate present would be resolved into acid phosphates, but the amount of calcium phosphate present in gastric juice is altogether insufficient to account for any appreciable part of its acidity.

While the subject was still in this vexed condition, Bidder and Schmidt's[10] classical work on digestion appeared, containing the results of Schmidt's experiments, to which reference has already been made. As

[1] *Annals of Philosophy*, July 1824.

[2] *Ann. de chim.*, Paris, 1835, tome lix. p. 348.

[3] Published with Beaumont's results, 1834.

[4] As is often the case in great discoveries, Prout seems not to have been much in time ahead of his fellows. Tiedemann and Gmelin state in the preface to their classical work, "Die Verdauung nach Versuchen," 1826 (while admitting Prout's priority), that independently they had found hydrochloric acid in distilling various gastric fluids, and a month later first saw Prout's publication. However, Prout was clearly ahead of them, both in the distillation method, and in its ingenious confirmation by analytical results, as described in the text.

[5] *Compt. rend. Acad. d. sc.*, Paris, 1844-5 ; "Leçons de physiol. exper. appliqué à la méd.," 1856, tome ii. p. 397.

[6] *Ber. d. Sächs. Gesellsch. d. Wissensch.*, Leipzig, 1847.

[7] Pelouse, *Compt. rend. Acad. d. sc.*, Paris, tome xix. p. 1227 ; Thomson, *Lond. Edin. and Dub. Phil. Mag.*, London, 1845.

[8] "Traité analytique de la digestion," 1843 ; *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1851, Bd. i. S. 97 ; 1858, Bd. i. S. 37.

[9] See p. 361.

[10] "Die Verdauungssäfte und der Stoffwechsel," Mitau u. Leipzig, 1852, S. 44.

the result of eighteen concordant analyses, Schmidt found that gastric juice always contained more hydrochloric acid than was sufficient to neutralise all the bases present, and that the excess of hydrochloric acid was alone sufficient to account for the entire acidity of the gastric juice.

Schmidt's course of procedure was as follows:—

The total chlorides in a weighed quantity (100 grms.) of gastric juice were precipitated and weighed as silver chloride in the usual fashion, by adding a drop or two of nitric acid followed by slight excess of silver nitrate solution. From the filtrate the excess of silver nitrate was removed by addition of pure hydrochloric acid, as silver chloride; and the filtrate, containing all the bases of the gastric juice, was evaporated to dryness, ignited, and the amount of each separate base in the ash determined by appropriate methods. In many cases the percentage of ammonia present was also determined in a different portion as ammonio-platinic chloride.

The amount of hydrochloric acid present, combined and uncombined, was found from the weight of the first silver chloride precipitate; the weight of chlorine necessary to combine with the weight of each base present was next calculated, on the assumption that *all* of each base was actually present as chloride; and by adding all these weights of chlorine the amount of chlorine (and hence hydrochloric acid) necessary to satisfy all the bases was determined. This was found to be considerably less than the total chlorine present; in fact, the difference in the two amounts represented very accurately the total acidity reckoned as hydrochloric acid.

The argument underlying Schmidt's experiments cannot be gainsaid, and as the experimental part of his work was confirmed by other observers,[1] there remained no choice but to accept the presence of hydrochloric acid in the stomach as proven. This view accordingly gained ground after the publication of his results, and is now universally accepted.

Although Schmidt's experiments demonstrate that there is an excess of hydrochloric acid in gastric juice, *uncombined with inorganic bases*, they do not show that this excess of acid is entirely uncombined. It is certain that if the excess of acid is in chemical combination with anything, the compound so formed is a very unstable one; this is shown by the ease with which the acid combines with fixed alkalies, and by the persistence of the acid reaction in spite of the combination. Still there are clear grounds for believing that the hydrochloric acid is in most cases combined loosely with some other body, most probably albumose or peptone, which are always present in traces in gastric juice. These reasons are as follows:—

1. Organic acids do not dissolve calcium oxalate, but a solution of hydrochloric acid in water, containing one part of acid in a thousand parts of water, does dissolve this compound. Now gastric juice does *not* dissolve calcium oxalate, from which Bernard and Barreswil[2] argued that the acidity of gastric juice is not due to hydrochloric acid. This difference in action on calcium oxalate of (*a*) a solution of hydrochloric acid in water, and (*b*) gastric juice, is, however, probably due to the presence of albumoses and peptones, which form a loose combination with the acid, of sufficient stability to prevent it from acting on calcium oxalate.

[1] Ch. Richet, "Le suc gastrique chez l'homme et les animaux," Paris, 1878, p. 32; Maly, *Ann. d. Chem.*, Leipzig, 1874, Bd. clxxiii. S. 227.

[2] Cl. Bernard, "Leçons de physiol. expér.," 1856, tome ii. p. 395.

2. Laborde[1] compared the inverting power of gastric juice on cane-sugar with that of a solution of pure hydrochloric acid in water, of equal acidity to the gastric juice, and under similar conditions. He found that the hydrochloric acid inverted much more rapidly than the gastric juice, which possessed much the same inverting power as a solution of lactic acid of equal concentration. He also found that gastric juice converted starch into grape-sugar and dextrin much more slowly than a solution of hydrochloric acid under similar conditions. On the other hand, Szabo[2] found that peptones do indeed interfere with the action of dilute hydrochloric acid on starch; but, contrary to Laborde, found that the action of gastric juice on starch lies in intensity much closer to that of hydrochloric than to that of lactic acid.

3. In treating of the digestive enzymes, it has been seen that these are much less injured by hydrochloric acid, in presence of albumoses and peptones, than by free hydrochloric acid alone, which shows that hydrochloric acid in presence of albumoses and peptones behaves as if it entered into combination with them.

4. Berthelot and Jungfleisch[3] showed that, when a substance which is soluble in each of two solvents, which are not completely soluble in each other, is shaken up with a quantity of both solvents, it divides itself between the two solvents so that the ratio of its concentrations in each is constant, and does not vary with the proportion of the two solvents used, nor the amount of soluble material used. This constant ratio they called the *coefficient de partage*, which may be rendered in English "coefficient of distribution."[4] For example, if succinic acid be well shaken up with water and ether, the concentration of succinic acid in the watery layer will always be about six times as great as in the ethereal layer, no matter, within wide limits,[5] what have been the quantities of ether, water, and succinic acid used; the coefficient of distribution is here six. Mineral acids are much more soluble in water compared with ether than are organic acids; accordingly the coefficients of distribution of the mineral acids for these two solvents are much larger than those of the organic acids.

Richet[6] made use of this property to test whether pure gastric juice contains only hydrochloric acid, or hydrochloric acid plus organic acids. He found that the coefficient of distribution was 137·1. To a portion of the same gastric juice he next added barium lactate, and found that the coefficient was reduced to 9·9, that of lactic acid is 8·8 to 11·0. This experiment shows that the acid first present was a mineral acid, which afterwards displaced nearly all the lactic acid from combination, so that in the second case the acidity was mainly due to lactic acid. Richet further added sodium acetate (*a*) to a solution of hydrochloric acid in water; (*b*) to gastric juice of equal acidity, and found that in the first case the coefficient was reduced to 1·7 (practically that of acetic acid, 1·4), while in the second case the coefficient was only reduced to 5 to 5·8. Richet supposes that this difference is due to the hydrochloric acid in the gastric juice being combined feebly with some other substance. When sodium acetate is added to hydrochloric acid alone, the base will be shared between the two acids in proportion to their mutual avidities for it, which are in the ratio of 1 to ·03. That is to say, about

[1] *Gaz. méd. de Paris*, 1874, Nos. 32–34, pp. 399, 411, 422.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 140.

[3] *Ann. de chim.*, Paris, 1872, Sér. 4, tome xxvi. p. 396. For a complete account of this subject, see Ostwald, "Lehrbuch der allgemeinen Chem.," Leipzig, 1891, Aufl. 2, Bd. i. S. 809.

[4] This term has been proposed by Gamgee, "Physiological Chemistry," vol. ii. p. 97, as well as "coefficient of repartition."

[5] The quantities of solvent must be so chosen, compared with the quantity of soluble substance, that the solutions are not too concentrated.

[6] "Le suc gastrique chez l'homme et les animaux, ses propriétés chemiques et physiologiques," Paris, 1878, p. 37.

97 per cent. of the base will be combined with the hydrochloric acid, and 3 per cent. with the acetic acid; or, otherwise, 3 per cent. of the hydrochloric acid will be free and 97 per cent. of the acetic acid, supposing that equivalent quantities of the two acids are present.[1] Such a mixture would possess only a slightly higher *coefficient of distribution* than acetic acid. But if the sodium acetate be added to hydrochloric acid, already feebly combined with something else, the power of the acid to combine with the sodium will be diminished, on account of the tendency to remain combined with this substance, and the amount of hydrochloric acid uncombined with sodium will be increased; this will remain to a greater extent in the watery layer, and on shaking with ether the *coefficient of distribution* will be much greater than that of acetic acid.

Richet found traces of leucine in the gastric mucous membrane, and believes, mainly on this ground, that the hydrochloric acid of the gastric juice is in combination with leucine. But there is no good reason for going so far afield to seek a partner for the hydrochloric acid; any substance in combination with the acid would produce such an effect as Richet obtained, and it is far more probable that the hydrochloric acid is in combination with the albumoses of the gastric juice than with leucine, especially as leucine has not been found in gastric juice, and hydrochlorate of leucine does not act as an acid to pepsin, as shown by the inability of a mixture of the two to digest proteids.[2]

This account of Richet's work has been placed here on account of the bearing of the latter part of it on the question of the combination of the hydrochloric acid, but the first part of it is also of great value in showing that *pure* gastric juice is practically free from organic acid.

Organic acids present during carbohydrate digestion.—Although organic acids are entirely absent in pure gastric juice, or at most are only present in traces, this is by no means the case during digestion, especially of food rich in carbohydrates.

The food passing into the stomach during a meal is alkaline in reaction, by reason of the saliva with which it is abundantly mixed; and in addition, during and after a meal a considerable quantity of saliva is swallowed by itself. As Beaumont[3] and others have shown, there is no secretion of acid gastric juice when the stomach is empty, and although active secretion begins with the arrival of the first portions of food in the stomach, some time must elapse before the alkaline reaction of the masses of food and saliva is neutralised by the acid of the gastric juice, and a reaction due to free hydrochloric acid established, after saturation of the soluble proteid of the food. This interval is exceedingly difficult to estimate, the delicate colour reactions for free hydrochloric acid being so deceptive in a heterogeneous fluid like the contents of a stomach; van de Velden[4] states that it varies from half an hour to two hours, and is on an average three-quarters of an hour. During this time conversion of starch by ptyalin goes on,[5] and in addition bacterial action begins with the production, from the carbohydrate part of the food, of lactic acid,[6] accompanied by traces of butyric and acetic acids.

[1] J. Thomsen, "Thermochemische Untersuchungen," *Ann. d. Phys. u. Chem.*, Leipzig, 1869–1871, Bde. cxxxviii.-cxliii.

[2] See Gamgee, "Physiological Chemistry," vol. ii. pp. 97-99.

[3] See article on "Mechanism of Gastric Secretion."

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 205.

[5] See under "Ptyalin," p. 329.

[6] According to Maly, the greater part of the lactic acid is the ordinary lactic acid of fermentation, but this is accompanied by a smaller quantity of sarcolactic acid, which may occasionally be much increased in amount, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1874, S. 156; *Ann. d. Chem.*, Leipzig, 1874, Bd. clxxiii. S. 227.

It was long believed that this action was due to the growth of the *Bacillus acidi lactici* on sugar only, either that of the food or that produced by the action of ptyalin on the starch of the food. Brücke [1] has shown, however, that starch can be also changed into lactic acid without conversion by ptyalin, by demonstrating that soluble starch, erythrodextrin and lactic acid, are found in the stomach of the dog after a meal containing boiled starch. Now the saliva of the dog contains no ptyalin, so that these products must be formed directly from starch. Traces of sugar are also found, and Brücke supposes that sugar is first formed by the action of the *bacterium* but immediately becomes converted into lactic acid by its further action. A similar change in starch paste takes place on standing in the air.

Goldschmidt [2] divides gastric digestion in the horse into four stages, which are, however, not sharply marked off, but merge into one another. (1) No proteolysis, acid reaction due to lactic acid. (2) Proteolysis and amylolysis proceed together, both lactic and hydrochloric acids present. (3) Stoppage of amylolysis in the middle part of the stomach, in this portion only hydrochloric acid, elsewhere lactic acid. (4) Stoppage of amylolysis everywhere; hydrochloric acid only present in all parts of the stomach. Ewald and Boas [3] describe a similar state of affairs in the healthy human stomach under normal conditions after a carbohydrate meal. In the first stage (from ten to thirty minutes after the meal) lactic acid alone is present; in the second, lactic and hydrochloric acids are present together, but the former rapidly disappears so soon as any free hydrochloric acid is present; and in the third stage, hydrochloric acid alone is present. This disappearance of the lactic acid is very interesting, as showing that it is rapidly absorbed in the stomach.

Other inorganic acids free in pure gastric juice besides hydrochloric acid.—It must not be assumed, from the usual mode of stating the results of quantitative analysis of gastric juice,[4] that hydrochloric acid is the only inorganic acid present in the gastric juice. All the phosphoric acid is not united, in the gastric juice, to calcium, magnesium, and iron to complete saturation, as usually set forth in such analytical results; nor are all the bases saturated by the hydrochloric acid, and only that amount of hydrochloric acid free, which is left over after so saturating them.[5] Suppose a solution in water of neutral chlorides is taken, say such a solution as the gastric juice would be, minus its free hydrochloric acid and its phosphates, and to this phosphoric acid is added. As soon

[1] Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1872, Bd. lxv. Abth. 3, S. 126; "Vorlesungen," Wien, 1885, Aufl. 4, Bd. i. S. 321. See also W. de Bary, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xx. S. 243.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 361. See also Ellenberger and Hofmeister, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1885, Bd. xv. S. 284, 301; 1886, Bd. xvi. S. 260, 261.

[3] *Virchow's Archiv*, 1885, Bd. ci. S. 325; 1886, Bd. civ. S. 271; Ewald, "Klinik der Verdauungskrankheiten," 1890, Bd. i. S. 83.

[4] See p. 350.

[5] This was merely an assumption made by Schmidt, in order to conclusively show that gastric juice contained an excess of hydrochloric acid above even *this* quantity. Fortunately, the excess of hydrochloric acid was sufficient to allow Schmidt to give this form of proof; but if the quantity of phosphates had been greater, or the excess of hydrochloric acid less, Schmidt's process might easily have yielded a negative result, and yet the gastric juice have contained free hydrochloric acid; indeed, the massed equivalent in chlorine of the total bases might have been greater than the total quantity of chlorine present, and still there might have been free hydrochloric acid present.

as the phosphoric acid passes into solution, it no longer remains present as free phosphoric acid, to the amount to which it has been added, but reacts with the other salts present in solution, displacing a definite amount of each metal from combination with chlorine, thus setting free hydrochloric acid and forming phosphates, so that there comes to be in solution free hydrochloric acid and free phosphoric acid, combined phosphoric acid, and combined hydrochloric acid (that is, chlorides and phosphates). When a polybasic acid, such as phosphoric acid, is present in solution, the matter is somewhat further complicated by there being certain steps between free acid and combined acid, namely, acid salts; these also are represented in the distribution of bases among the acids, so that there are in solution free acids, acid salts, and neutral salts. In pure gastric juice, then, the acidity is in chief due to hydrochloric acid, but also in part to acid phosphates and phosphoric acid, and the amount of each of these free is perfectly determinate, and depends upon the amount of each base and each acid present. For one fixed distribution only can there be chemical equilibrium in the solution; the introduction of any salt, acid, or base into the solution will alter this equilibrium, and a new distribution to suit the new conditions will occur, giving rise again to equilibrium.

The facts stated above follow directly from Thomsen's[1] "avidity law." Thomsen arrived at this law by comparing the amount of heat set free when an equivalent weight of a base unites with a mixture of equivalent weights of two different acids, with the amount set free when it combines with each acid separately.[2] The law is that no acid in solution is combined with the bases present, to the complete exclusion of other acids, however weak (as it is popularly expressed), which may be simultaneously present in the solution; but the acids share the bases, according to their different avidities. Thomsen worked out a number of avidity coefficients. Those of the organic acids are much smaller than those of the inorganic acids. Thus, taking the avidity coefficient of hydrochloric acid as unity, that of oxalic acid is ·25, tartaric acid ·05, acetic acid ·03. These coefficients mean, for example, that if one equivalent each of sodic hydrate, of hydrochloric acid, and of oxalic acid, be mixed in solution together, four-fifths of the base is combined with the hydrochloric acid and one-fifth with the oxalic acid, and consequently one-fifth of the hydrochloric acid is free and four-fifths of the oxalic acid.

Maly[3] has also shown *qualitatively*, by a method of diffusion, that this displacement of a strong acid (*i.e.* acid with a large avidity coefficient) by a weak acid (acid with a small acidity coefficient) takes

[1] "Thermochemische Untersuchungen," *Ann. d. Phys. u. Chem.*, Leipzig, 1869–71, Bde. cxxxviii.–cxliii.

[2] Let a be the amount of heat in heat units developed when, say, one equivalent of NaOH in grammes combines with one equivalent of HCl, and b that when it combines with an equivalent of HNO_3, c that when it partially combines with a mixture of one equivalent of HCl and one equivalent of HNO_3, also let x be the fraction which combines with HCl. Then, since a is the amount of heat set free when a whole equivalent of NaOH unites with HCl, $a\,x$ will be that set free when the fraction x combines; similarly $b\,(1-x)$ will be the amount set free by the combination of the fraction $(1-x)$ with HNO_3; the sum of these two must equal c, the amount of heat actually observed; therefore $a\,x + b\,(1-x) = c$, from which x and $1-x$ can be determined. Their ratio is the measure of the avidity of the two acids for combining with the base.

[3] *Ann. d. Chem.*, Leipzig, 1874, Bd. clxxiii. S. 250; *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1874, Bd. lxix. Abth. 3, S. 251; *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 174.

place in solution. He dissolved sodium chloride and lactic acid together in water, placed the solution in the bottom of a cylindrical vessel, and then carefully poured a layer of distilled water on the top. After some days, part of the upper layer was removed and analysed; it was found to contain more than sufficient chlorine to balance all the sodium present; that is to say, it contained free hydrochloric acid. Similar results were obtained with a mixture of monosodium phosphate, and other acid salts, in common solution with sodium chloride.

These results of Thomsen and Maly will be again referred to in discussing the mode of origin of hydrochloric acid. They are introduced here to show that any weaker acids in gastric juice along with the hydrochloric acid *must* in part be uncombined. Any organic acids present during digestion will also be in part free and in part combined, and as these have very small avidities compared with hydrochloric acid, they will be almost completely free. This has a bearing of some importance. Any organic acids formed in the stomach by bacterial action on carbohydrates will be found as free acids, and will not reduce the amount of free hydrochloric acid,[1] but salts of organic acids entering the stomach with the food will reduce the amount of acidity due to free hydrochloric, because, from the organic salts, free acids will be formed, by hydrochloric acid combining with their bases.

Source of the hydrochloric acid.—The only possible source of chlorine lies in the chlorides of the food, and from this either directly, or indirectly through the blood, the hydrochloric acid must necessarily have its origin. That the chlorides present in the blood plasma are the source of the acid, has been experimentally proved by Voit[2] and Cahn.[3]

Following a method first used by Voit, Cahn fed dogs exclusively on meat which had previously had all its salts extracted by boiling it repeatedly with distilled water. An animal fed in this manner continues to excrete a diminishing quantity of chlorides in the urine for a period varying from two to five days. After this only traces of chlorides are found in the urine, but the tissues and blood still cling on to their necessary minimum quantity of chlorides, digestion goes on, and the animal lives. At this period, if the contents of the stomach are washed out with distilled water, the secretion is found to contain free acid and to possess digestive power. If now the animal's reserve stock of chlorine be still further reduced by administering diuretics, such as potassium nitrate, which cause some additional chlorides to be excreted; or if free hydrochloric acid be repeatedly removed by pumping out the contents of the stomach with the aid of distilled water, a condition is finally reached in which the stomach secretes a completely neutral fluid, which is altogether inactive so long as it is neutral, but quickly digests fibrin if 1 part per 1000 of hydrochloric acid be added to it. When this stage is reached the animal rapidly fails; but if a small quantity of sodium chloride be now given to it, it rapidly recovers, and soon becomes in every respect normal.

This experiment also shows that the secretion of pepsin is independent of that of acid, and that in the absence of hydrochloric acid no

[1] In fact will slightly increase it by combining to a certain extent with the bases of the chlorides.

[2] *Sitzungsb. d. k.-bayer. Akad. d. Wissensch. zu München*, 1869, Bd. ii. S. 483. See also M. Gruber, *Beitr. z. Physiol. C. Ludwig z. s. Geburtst.*, Leipzig, 1887.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 522.

lactic acid or other organic acid is formed, which disproves the theory that lactic acid is first formed and then decomposes sodium chloride, so forming free hydrochloric acid.[1]

It may here be pointed out that experiments have been made by Nencki and Schoumova-Simanowsky[2] to ascertain the possibility of replacing the chlorine by other halogens, so as to form hydrobromic or hydriodic acids. These experiments were performed on dogs operated on by Pawlow's method,[3] and the animals were fed with food in which sodium chloride was as far as possible absent. Some had added to their food sodium bromide, others sodium iodide. The administration of sodium bromide resulted in the animals becoming so ill after a week or so that the experiments had to cease. The gastric juice was secreted as before, but the hydrochloric acid was largely replaced by hydrobromic acid. In the case of those dogs to which sodium iodide was administered, though less general disturbance resulted from the administration than was the case with sodium bromide, yet the amount of hydriodic acid replacing hydrochloric acid was very small.

Reciprocity between the secretion of hydrochloric acid and the reaction of the urine.—That the hydrochloric acid of the gastric juice is formed from the chlorides of the blood plasma, is likewise shown by Maly's[4] observation that at the same period after a meal at which the secretion of gastric juice is at a maximum, the acidity of the urine is at a minimum, and may be replaced by an alkaline reaction. One function of the kidneys is to preserve unaltered in degree the alkalinity of the blood. If now neutral salts, such as sodium chloride, be removed from the blood, split up in some manner by the agency of the gastric gland cell into hydrochloric acid and sodic hydrate, of which the hydrochloric acid is sent towards the stomach cavity, while the alkali is expedited in the opposite direction back to the blood stream, it follows that the alkalinity of the blood will be increased. Hence, to preserve equilibrium, the kidneys must excrete a less proportion of acid salts, or, if the rate of increasing alkalinity of the blood demands it, must separate an alkaline fluid from the blood. This is experimentally found to be the case. Under ordinary circumstances, the kidneys preserve the constant value of the alkalinity of the blood, by excreting phosphates of the alkalies so proportioned that the reaction is acid, but during active digestion, 2 to 4 hours after a full meal such as dinner, the relative amounts of bases and phosphoric acid are so altered that the reaction becomes neutral or faintly alkaline, or, as it is often commonly but not very exactly expressed, in the first case monosodic phosphate (NaH_2PO_4) is secreted with acid reaction; in the second, disodic phosphate Na_2HPO_4 with alkaline reaction.[5]

Theories as to the mode of origin of the hydrochloric acid.—Many ingenious theories have been proposed to account for the specific function of the gland cells of the stomach, of splitting up such a stable substance

[1] See p. 352.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1894, Bd. xxxiv. S. 313.

[3] See article on "Mechanism of Gastric Secretion."

[4] *Ann. d. Chem.*, Leipzig, 1874, Bd. clxxiii. S. 232. See also Quincke, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, Bd. iv. S. 241; Stein, *ibid.*, 1876, Bd. vi. S. 161.

[5] The reaction will really vary according to the relative amounts of base and acid present. Monosodic phosphate alone dissolved in water has an acid reaction, disodic phosphate similarly has an alkaline reaction, and mixtures in varying proportions can have acid, neutral, or alkaline reaction. In a complex mixture such as urine, no one can say to what the reaction is due, but only that there is an excess of alkali or acid.

as sodium chloride, of forming such a strong acid as hydrochloric acid is in the face of the alkalinity of the blood, and of determining an alkaline stream towards the blood and an acid stream towards the lumen of the gland.

The oldest theory was, that the process was an electrolytic one. Blondlot[1] supposed that by electric agency sodium chloride in the stomach wall was broken up into sodic hydrate and hydrochloric acid (in the language of to-day, hydrolysed, $NaCl + H_2O = NaHO + HCl$). The free acid then, for the most part, acted on the calcium phosphate of the blood, forming acid phosphate and a trace of phosphoric acid, while a trace of hydrochloric acid also remained free. To such a mixture of acid substances (mainly acid calcium phosphate) he ascribed the acidity of gastric juice. He electrolysed tricalcic phosphate, suspended in a solution of sodium chloride, and claimed to have obtained such products as his theory demands. Brücke[2] considered that the energy required came from transformation of nervous energy, modified to this purpose, and, admitting that the details are not explicable, compared the effect to others called forth by nerve impulses, such as the electric effects in the electric end-organ of some fishes. He also considered the secretion of acid more analogous to electrolysis than to any other known process. Lussana[3] supposed that in the glands of the stomach a decomposition of the salts of the plasma took place, and that the preponderating part of the free acid of the gastric juice was hydrochloric, simply because by far the greater part of the salts of the plasma are chlorides. He tried to test his theory by intravenous injection of salts not present in quantity in blood plasma, such as sulphates and phosphates. He did not, however, obtain the corresponding acids in the gastric juice, except in the case of borax and tartar emetic, after injection of which traces of boric and tartaric acids respectively were found in the gastric juice.

Buchheim[4] suggested that the chlorides of the plasma combined with the proteid, so that the metal combined with one proteid molecule and the acid radicle with another; the latter combination being absorbed by the acid-secreting cells and broken up there into proteid and acid.

These older theories can at best be only regarded as mere speculations; there is absolutely no experimental proof of them. Nor can we lay claim at the present day to a complete knowledge of the process of secretion of hydrochloric acid. Only thus far the progress of physical chemistry, and a more exact knowledge of the laws of solutions, has brought us, that we no longer need look upon the production of hydrochloric acid by the animal organism as a chemical wonder. The secretion of hydrochloric acid is still a mystery as great as the secretion of pepsin or any other product of cell activity, but no greater.

To the chemist, before the thermochemical work of Thomsen, and the diffusion experiments of Maly already described,[5] and when he was acquainted with no other means of setting free hydrochloric acid from its salts than the electric current or displacement by a stronger acid such as sulphuric acid, the occurrence of hydrochloric acid in the gastric

[1] "Traité analytique de la digestion," Nancy et Paris, 1843; *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1851, Bd. i. S. 97; 1858, Bd. i. S. 37. See also Ralfe, *Lancet*, London, 1874, vol. ii. p. 29.

[2] "Vorlesungen," Wien, 1885, Aufl. 4, Th. 1, S. 307.

[3] *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1862, Bd. i. S. 110.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 332.

[5] See p. 357.

juice was an unsolvable riddle. But when Thomsen had shown that the weakest acid is in some measure capable of displacing the strongest from its salts, and Maly that by a simple process of diffusion this strong acid may be afterwards separated, the subject assumed a different aspect. It was no longer necessary for the cell to be endowed with some force of sufficient intensity, to directly break up such stable substances as the alkaline chlorides. All that was necessary was that the cell should be able from the organic material at its disposal to form an organic acid, and afterwards to rapidly excrete the small fraction of hydrochloric acid formed by the interaction between this organic acid and the neutral chlorides, so that a fresh quantity of hydrochloric acid may be formed by the mass action of the remainder of the organic acid on the remainder of the chlorides. The organic salts so formed can then decompose by cell activity into organic acid and base again, and the base be returned to the blood stream. Since gastric juice is not accompanied by an organic acid, this must be retained in the cell and induce a continuous cyclic change. It is thus possible, with the aid of the new facts of physical chemistry, to see that the process of secretion of hydrochloric acid can be reduced to the same level as that of the secretion of any organic material.

This, however, is but a small portion of the entire problem. As Bunge says: "In the appearance of the free hydrochloric acid lies nothing puzzling. Puzzling only is the power of the epithelial cell to send the hydrochloric acid freed from the sodium chloride streaming always in one direction towards the lumen of the gland, and the sodium carbonate [1] simultaneously formed always back in the opposite direction towards the lymph and blood channels. But such a puzzle we meet everywhere in the living tissue. Every cell possesses the power to dispose of material in a suitable manner, attracting or repelling it and sending it streaming in different directions." [2]

Maly's theory.—Maly has attempted to build on a purely physical basis a theory of the formation of hydrochloric acid from the chlorides of the blood, of which the following are the outlines: [3]—

1. There are no *theoretically* alkaline salts in the blood. Blood plasma owes its alkalinity to two *theoretically* acid salts, di-sodic phosphate (Na_2HPO_4), and sodium bicarbonate ($NaHCO_3$); besides these two acid salts plasma contains excess of carbonic acid.

2. Disodic phosphate in presence of calcium chloride forms some free hydrochloric acid, thus:—$3CaCl_2+2Na_2HPO_4=Ca_3(PO_4)_2+4NaCl+2HCl$.[4]

Chiefly from the facts above stated, Maly supposes that by the interaction of these *theoretically* acid salts of the plasma, on the chlorides present with them in solution, traces of hydrochloric acid are formed; these traces of hydrochloric acid are rapidly removed, on account of the high diffusibility of hydrochloric acid,[5] by the gland-cells which act as a

[1] Bunge is considering the hydrochloric acid as set free by the action of carbonic acid.

[2] Somewhat freely translated from Bunge, "Lehrbuch der physiol. Chemie," Leipzig, 1894, Aufl. 3, S. 148.

[3] Abstracted from Maly, Hermann's "Handbuch," Bd. v. (2), S. 66.

[4] R. Pribram, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1871, Bd. i. S. 107; Gerlach, *ibid.*, 1873, Bd. iii. S. 109.

[5] Graham has shown that the free acids diffuse more rapidly than their salts; HCl diffusing thirty-four times as rapidly as NaCl. Graham was also the first to show that, by diffusion of acid potassium sulphate, sulphuric acid was obtained in the dialysate, while normal sulphate remained behind.

perfect diffusion[1] apparatus; on the removal of the hydrochloric acid, fresh acid is formed by further mass action on the chlorides. The kidneys or sweat glands probably do not so secrete hydrochloric acid, because they are not such perfect diffusion arrangements as the gastric glands, and cannot bring about such a molecular separation as the latter.

Objections to Maly's theory.—1. Modern work has shown that the alkaline reaction of *theoretically* acid salts is probably due to a hydrolysis taking place on solution. Thus on dissolving sodium bicarbonate there are formed sodic hydrate and carbonic acid ($NaHCO_3 + H_2O = NaOH + H_2CO_3$); and the sodic hydrate being a powerful base, and the carbonic acid a weak acid, one equivalent of the base more than balances two of the acid, and the reaction is alkaline. On the other hand, when acid potassium sulphate is dissolved, there is one equivalent in solution of a strong base, and two equivalents of a strong acid, and the reaction is acid. Such an hydrolysis of phosphates of the alkalies also takes place. Trisodic phosphate yields an equivalent of base to one of acid, and the reaction is intensely alkaline; disodic phosphate yields only two equivalents of base to three of acid, but the reaction is still alkaline; while monosodic phosphate yields but one equivalent of base to three of acid, and at last the reaction is acid. A mixture of mono- and disodic phosphates in proper proportion would be neutral. In fact, after these salts are dissolved, they no longer exist as such, but there are present in solution bases and acids in certain concentrations, and the reaction of the solution will depend on which of these acts most strongly on the indicator. Now the hydrolysing effect on the neutral salts, chlorides, etc. (if such are also present in solution), of these so-called acid salts must closely resemble their effect on the indicator.

Whether there will be a tendency to formation of hydrochloric acid or not from sodium chloride, will be determined by whether the attraction of the acids (phosphoric and carbonic) for the base is greater or less than the attraction of the bases for the hydrochloric acid. The reaction of the solution of phosphates and carbonates in the plasma is alkaline, which shows that the latter is the case, and that, therefore, there will be no hydrochloric acid formed.

2. The continuous formation of hydrochloric acid by a reaction between disodic phosphate and calcium chloride is impossible, because it necessitates the formation of insoluble tricalcic phosphate, and as the supply of calcium chloride is small, must soon stop.

3. Even if it be admitted that there are traces of hydrochloric acid in the blood, there is no reason, if the process be purely one of diffusion, why it should not go on continuously. This it does not do, but ceases when digestion is not going on, and when digestion begins is secreted in such amount that no mere physical diffusion could bring it through the epithelial cells fast enough; not to speak of separating it from a fluid in which it is supposed to be present in traces only.[2]

[1] By a perfect diffusion apparatus (*vollkommener Diffusions-apparat*) Maly seems to mean here semipermeable membrane; that is, an arrangement permeable to the hydrochloric acid and not to the other dissolved substances.

[2] Gastric juice contains at least 2 parts per 1000 of hydrochloric acid; the amount of hydrochloric acid formed by mass action in a solution of 6 parts per 1000 of sodium chloride, and a still smaller quantity of monosodium phosphates, no one has ever attempted to measure, but it must be many thousand times less than this; so that not only must the hydrochloric acid diffuse with a tremendous velocity, but it must get infinitely more concentrated in the process of diffusion, which, under *purely* physical conditions, so far as we know them, is an utter impossibility.

These facts indicate that the formation of hydrochloric acid is a process going on in the cell, that the acid is a cell secretion, and not a diffusate from the blood plasma.

Gamgee's modification of Maly's theory.—Gamgee,[1] while retaining the supposition that the hydrochloric acid is formed by the action of the alkaline phosphates on the chlorides, removes the seat of action from the blood to the parietal cells. He supposes that these cells possess a peculiar selective absorption for the phosphates of sodium, both alkaline and acid, and for chlorides, and that within the cell there occur the same reactions between these substances as occur *in vitro* when they coexist in solution. One of the products of the reaction will then be hydrochloric acid, which, in virtue of its high power of diffusion, will pass, as soon as formed, into the secretion of the gland. This supposition is certainly a step in the right direction, in so far as it brings the seat of action to the cell—a much more probable place than the blood—but, on the other hand, it assumes a good deal, without overcoming many of the objections to Maly's theory. Thus, selective absorption, of both alkaline and acid phosphates (probably di- and mono-sodium phosphates) is assumed. Unless these are also assumed to be absorbed in such proportions that the reaction of the cell contents becomes acid, no formation of hydrochloric acid will take place, for, under merely physical conditions, no such formation can be demonstrated *in vitro*.

Unless, again, the substances selectively absorbed are kept out of action in some equally obscure manner by cell activity, there is no reason why the secretion of acid should not be continuous; and if absorption of phosphates and chlorides only begins at the commencement of digestion, it is not easy to see how the traces of hydrochloric acid, formed by such interactions, can keep pace with the demand then made for hydrochloric acid.

Lastly, there is no experimental evidence that there is any such selective absorption of phosphates and chlorides by the parietal cells. And if a purely physical theory is to be abandoned, and a specific functional activity of the cell invoked, there remains no reason for adhering to theories which have been evolved on a purely physical basis.

It is easier, and more in accordance with our notions regarding the secretion of other substances, to suppose that the hydrochloric acid is formed by cell activity in some metabolic process, from the chlorides and organic matters at its disposal. There are an infinite variety of such processes capable of taking place, under the varying conditions of cell life. It is true we do not know the details of these, nor why such processes take place under certain given conditions; nevertheless we see the end-results, and there is no reason why hydrochloric acid should not also be the end-product of such a cell metabolism rather than the product of a kind of specialised diffusion.[2]

[1] "Physiological Chemistry," 1893, vol. ii. p. 113.

[2] Hammarsten, "Lehrbuch der physiol. Chem.," Wiesbaden, 1895, Aufl. 3, S. 242. See also Heidenhain, Hermann's Handbuch," Bd. v. (1), S. 151. One such possible process is the formation in the cell of an organic acid which does not diffuse away, but is retained in the cell and exercises a continuous action on the chlorides, forming hydrochloric acid which the cell actively excretes. Another possibility would be the formation during rest of an organic chlorine-containing substance, while the base combined with carbonic acid passed into the blood, and the subsequent breaking up during activity of this chlorine-compound yielding hydrochloric acid. There are indeed many courses which such a cell-metabolism might take yielding hydrochloric acid as an end-result. See also Bunge, "Lehrbuch der physiol. Chemie," Leipzig, 1894, Aufl. 3, S. 149.

Function of the hydrochloric acid.—One obvious purpose of the hydrochloric acid of the gastric juice is to confer activity on the pepsin accompanying it, which is only active in an acid medium. But, as Bunge[1] points out, the establishment of an acid reaction is not necessary for proteid digestion. In the pancreatic juice another proteolytic ferment, trypsin, is found, which acts most powerfully on proteids in an alkaline medium. A much more important function of the hydrochloric acid lies, according to Bunge, in its powerful action as a disinfectant and germicide, in destroying bacteria introduced with the food. In this manner the formation of decomposition products, and the disturbance thereby produced in the normal course of digestion, is prevented, and also in many cases the animal is preserved from the attacks of pathogenic bacteria by the destruction of these or their spores.

Modern research has, in fact, led to the remarkable result, that the average amount of hydrochloric acid found in the gastric juice just about coincides with that which is found experimentally to be required to stop the growth of most fermentative organisms and many pathogenic bacteria.[2]

Spallanzani[3] first called attention to the powerful preservative action of gastric juice, and not only showed that gastric juice prevented putrefaction, but that it stopped putrefaction which had already commenced. This he showed by feeding dogs on pieces of flesh which had commenced to putrefy. After a short interval of gastric digestion the flesh lost all putrefactive odour.

The action of the gastric juice on the bacilli of tubercle and splenic fever has been investigated by Falk,[4] and by Frank.[5] Falk found that the bacillus of splenic fever (*B. anthracis*) is easily destroyed by gastric juice, but that its spores escape destruction, and that the tubercle bacillus is unaffected by gastric juice. Frank completely confirms these results, and both observers are agreed that the gastric juice is incapable of making any very effectual resistance to infection of the organism by these pathogenic bacteria. The comma bacillus of cholera, however, is readily destroyed by gastric juice or dilute hydrochloric acid.[6] Cholera cannot be communicated by the mouth in healthy animals; but, after washing out the stomach with alkaline solutions, symptoms resembling those of cholera follow introduction of a pure culture of the cholera bacillus, as is also the case when this is introduced into the intestine.

The acetic and lactic fermentations are stopped by mere traces of *free* hydrochloric acid, while acid combined with proteid is ineffectual. According to Cohn, this action is due to the free acid decomposing the alkaline phosphates, which are necessary for the growth of the bacteria.[7]

Qualitative tests for free hydrochloric acid in gastric juice.—The many colour tests for detecting the presence of free hydrochloric acid in gastric juice, in contradistinction to organic acids, are all more or

[1] "Lehrbuch der physiol. Chemie," 1894, Aufl. 3, S. 141–145.

[2] Sieber, *Journ. f. prakt. Chem.*, Leipzig, 1880, Bd. xix. S. 433; Miquel, *Centralbl. f. allg. Gsndhtspflg.*, Bonn, 1884, Bd. ii. S. 403. See also Ziemke, Inaug. Diss., Halle, 1893; Mester, *Ztschr. f. klin. Med.*, Berlin, 1894, Bd. xxiv. S. 441; Schmitz, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xix. S. 401.

[3] "Expériences sur la digestion," Traduit par Senebier, Geneva, 1784.

[4] *Virchow's Archiv*, 1883, Bd. xciii. S. 177.

[5] *Deutsche med. Wchnschr.*, Leipzig, 1884, No. 20, S. 309.

[6] Nicati and Lietsch, *Rev. scient.*, Paris, 1884, p. 658; *Compt. rend. Acad. d. sc.*, Paris, 1884, tome xcix. S. 928; Koch, *Deutsche med. Wchnschr.*, Leipzig, 1884, No. 45, S. 725.

[7] *Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xiv. S. 75.

less influenced by the presence of proteid or peptone, and cannot be much depended on for proving the entire *absence* of hydrochloric acid. The quantity of organic acid required to give the reaction in each case is much in excess of that present in the stomach, so that if the test gives a positive result this may usually be relied upon.

The best of these reagents are the following:—(*a*) *Gunzberg's reagent*,[1] which consists of 2 parts of phloroglucinol, 1 part of vanillin, and 30 parts of absolute alcohol. A few drops of this reagent and a few drops of filtered gastric juice are evaporated to dryness together, when, if free hydrochloric acid be present, a carmine-red mirror or carmine-red crystals are obtained. The test is unaffected by organic acids, but does not succeed in the presence of proteids or leucine; it is said to detect 1 part of free acid in 20,000. (*b*) *The tropæolin test.*—Drops of a saturated solution of tropæolin in methylated spirit are allowed to evaporate on porcelain; to the stain so left a drop of the solution to be tested is applied, and the drop is evaporated at 40° C. In the presence of hydrochloric acid the result is a violet stain. The test has about the same delicacy as Gunzberg's, and is subject to the same objections. (*c*) *Reoch's test*[2] consists of a mixture of citrate of iron and quinine, and of potassium sulphocyanide. This is coloured red by a trace of a mineral acid, but not by dilute solutions of organic acid. Szabo[3] has modified this test into a quick, colorimetric quantitative method. He finds the Reoch test a satisfactory one, unaffected by chlorides, peptones, or the usual amount of lactic acid present in gastric juice. (*d*) *Congo-red* is strongly recommended by Gamgee,[4] either in aqueous solution, or as test paper made by saturating filter paper with it, and then drying. Traces of hydrochloric acid turn it an *intense blue*, while organic acids give a *violet tint.*

Gentian-blue, methylaniline-violet, malachite-green, and benzo-purpurin are other reagents which have been recommended as colour tests for traces of free mineral acids.

Quantitative estimation of the free hydrochloric acid of the gastric juice.—Mörner and Sjoqvist's method.[5]—This method consists essentially in converting all the acids present into barium salts by shaking up with barium carbonate, drying, incinerating, and extracting thoroughly with warm water. In the process of incinerating, the barium salts of the organic acids which may have been present are destroyed and barium carbonate is reformed; the barium chloride formed from the hydrochloric acid alone dissolves afterwards, and gives, by estimating the barium, a measure of the amount of hydrochloric acid present. Using litmus as an indicator, 10 c.c. of the gastric juice is neutralised with finely-powdered barium carbonate in a platinum evaporating dish. The mixture is dried on the water bath, the residue incinerated, the ash powdered, extracted with as little warm water as possible, and finally filtered. The filtrate should measure about 50 c.c. To this filtrate an equal volume of absolute alcohol is added, and then three or four drops of a solution containing 10 per cent. each of sodium acetate and acetic acid. Into this solution a standard solution of potassium bichromate, containing 8·5 grms. per litre, is run from a burette until all the barium is precipitated. The alcohol added aids the precipitation, and the acetate solution prevents the precipitation of calcium salts or the formation of any free hydrochloric acid. "Tetra

[1] *Chem. Centr.-Bl.*, Leipzig, 1887, S. 1560.

[2] *Journ. Anat. and Physiol.*, London, 1874, vol. viii. p. 274.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 152.

[4] "Physiological Chemistry," London, 1893, vol. ii. p. 94, where a full account of these colour tests may be found.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 1. See also Sjoqvist, *Skandin. Arch. f. Physiol.*, Leipzig, 1895, Bd. v. S. 277, where a full history of this subject is given, and a bibliography of over 150 memoirs on the subject.

paper"[1] is used as an indicator; this turns a deep blue when the end of the reaction is reached.

Leo's method.[2]—In this method two determinations are made.—First, of the total acidity by titrating 10 c.c. of the gastric juice, after the addition of 5 c.c. of a concentrated solution of calcium chloride, with decinormal sodic hydrate solution, using litmus as an indicator. Secondly, the amount of acidity due to acid phosphates is similarly determined in a fresh portion of the gastric juice, after removing the acidity due to free acid by shaking up with finely-powdered calcium carbonate. The difference gives the amount of acidity due to free acid.

Toepfer's method[3] consists in titrating 5–10 c.c. of the gastric juice against decinormal caustic soda with different indicators—(*a*) with phenolphthaleïn, (*b*) with alizarin, (*c*) with dimethylamido-azobenzol. The first titration gives the total acidity (consisting of free hydrochloric acid, hydrochloric acid combined with proteid, and organic acids); the second gives free hydrochloric acid, plus organic acids; the third, free hydrochloric acid only. Thus three equations are given for the determination of three unknown quantities. The method had been tested by Mohr with favourable results, and has the advantage of rapidity.

Qualitative tests for lactic acid.—1. Uffelmann's[4] test consists of an amethyst blue-coloured solution made by adding a trace of ferric chloride to a 1 per cent. solution of carbolic acid. A trace of lactic acid added to this causes it to turn yellow; hydrochloric acid only decolorises it, and must be present in relatively large quantity to do so. The test is most safely applied by filtering the contents of the stomach, extracting the filtrate with ether, distilling off the ether, extracting the residue with water, and *adding this to a small quantity of the reagent.* The test shows with 1 part of lactic acid in 10,000. 2. A *very* dilute solution of ferric chloride, possessing only a trace of colour, is much deepened in colour on the addition of a mere trace of lactic acid.

Pancreatic Juice.

Normal pancreatic juice is difficult to obtain in quantity, on account of the inflammatory changes occurring in the gland, in consequence of the operation of inserting a cannula into the duct.[5] The fluid obtained from a fistula of the pancreatic duct in an animal is quite different, according to whether it is collected soon after the operation, during the first two or three hours, or after the lapse of a day or two. The fluid secreted during the first few hours is rich in solids, and is secreted very slowly; that flowing from a permanent fistula is poor in solids, and is much more copious. The temporary secretion probably resembles the natural pancreatic juice much more closely than the permanent secretion.

[1] Paper impregnated with paraphenylendiamine. For modifications of this method see Fawitsky, *Virchow's Archiv*, 1891, Bd. cxxiii. S. 292; von Jaksch, "Klin. Diagnostik innerer Krankheiten," 1892, Aufl. 3; Boas, *Centralbl. f. klin. Med.*, Bonn, 1891, Bd. xii. S. 33; Kossler, *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xvii. S. 91.

[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1889, Bd. xxvii. S. 481.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xix. S. 104; Mohr, *ibid.*, S. 647.

[4] *Ztschr. f. klin. Med.*, Berlin, 1884, Bd. viii. S. 392.

[5] The first to make a pancreatic fistula was de Graaf, 1664. For modern methods see Cl. Bernard, "Leçons de physiologie expérimentale," Paris, 1856, tome ii. p. 180; Bernstein, *Arb. a. d. physiol. Anst. zu Leipzig*, 1869; Heidenhain, Hermann's "Handbuch," Bd. v. (1), S. 177; Rachford, *Journ. Physiol.*, Cambridge and London, vol. xii. p. 80; Vassiliew, *Arch. d. sc. biol.*, St. Pétersbourg, 1893, tome ii. p. 219; Foderà, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1896, Bd. xvi. S. 79; Lewin, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. For further details see article on "Mechanism of Pancreatic Secretion."

A temporary fistula should be made two or three hours after a meal, and the fluid collected during the next two or three hours. The greater number of such fistulæ have been made on dogs. The fluid obtained is clear like water, but of a slimy, syrupy consistency; it becomes still more viscid as it cools, and undergoes at 0° C. a true coagulation, separating into a gelatinous and a fluid portion. Its specific gravity is about 1·030. It contains in suspension white corpuscles, which exhibit sluggish amœboid movements. It is alkaline in reaction, the alkalinity being equal to 0·2–0·4 per cent. of NaHO, but the first few drops secreted may be acid. The alkalinity is commonly said to be due to carbonates and phosphates of sodium. The fluid is rich in proteid, froths on shaking, and on heating to 75° C. coagulates to a solid white mass. If kept warm for some time, its proteids become peptonised by the trypsin present with them. On dropping into water a precipitate is formed, which is soluble in dilute saline or acids. Alcohol gives an abundant flocculent precipitate, mostly soluble in water, and consisting of the proteid and enzymes. Leucine is present in traces, but not tyrosine. Similar secretions have been obtained from many other animals; the pancreatic juice of herbivora (rabbit, ox, and sheep) contains much less proteid than that of carnivora, but is in other respects similar.

The permanent secretion sets in at a variable period, from a few hours to some days after the operation. It is very similar to the temporary secretion, except in containing much less organic matter, and in having in consequence a much lower specific gravity, 1·010–1·011.

Quantitative chemical composition.—The following table gives the results of analyses of both temporary and permanent secretions of dog's pancreatic juice by C. Schmidt:[1]—

1. Analyses of Temporary Secretion, obtained directly after the Operation.			2. Analyses of Secretion, obtained from Permanent Fistulæ.		
	a.	*b.*	*a.*	*b.*	*c.*
Water	900·8	884·4	976·8	979·9	984·6
Total solids . . .	99·2	115·6	23·2	20·1	15·4
Organic matter . .	90·4	...	16·4	12·4	9·2
Ash	8·8	...	6·8	7·5	6·1

3. Composition of the Ash (in parts per 1000 parts of Pancreatic Juice).

	(*a*) From Temporary Secretion.	(*b*) From Permanent Secretion.
Soda (Na_2O)	0·58	3·31
Sodium chloride	7·35	2·50
Potassium chloride	0·02	0·93
Earthy phosphates with traces of iron . .	0·53	0·08
Trisodic phosphate (Na_3PO_4)	...	0·01
Lime (CaO) and Magnesia (MgO) . . .	0·32	0·01

[1] Quoted from Maly, Hermann's "Handbuch," Bd. v. (2), S. 189.

These results show that, even in the same form of fistula, the amount of total solids and of organic matter is a very variable quantity. This is also shown by the results obtained by others. In the dog, Bernard found the total solids in temporary secretion, 86 to 100 per 1000; Tiedemann and Gmelin, 87 per 1000; Skrebitzki, 23 to 56 per 1000; in the sheep, Tiedemann and Gmelin, 36 to 52 per 1000; in the horse, Hoppe-Seyler, 8·88 organic, 8·59 inorganic, per 1000; in the rabbit, Heidenhain, 17·6 per 1000; in the sheep, Heidenhain, 14·3 to 36·9 per 1000.

Very few analyses of human pancreatic juice have been made, and it has never been obtained under quite normal conditions. Herter[1] obtained pancreatic juice, containing all three ferments, from an enlarged duct, due to carcinoma of the duodenum, which contained per 1000 parts, 24·1 parts of total solids, 17·9 parts of organic matter, 6·2 parts of ash. Zawadski[2] has more recently published an account of human pancreatic juice, obtained from a pancreatic fistula, remaining after removal of a pancreatic tumour. This sample resembled in composition those obtained from temporary fistulæ in animals, much more closely than Herter's sample; it possessed a powerful digestive action, and probably was an almost normal secretion. It contained, per 1000 parts, 135·9 of total solids, 92 parts of proteids, 3·4 parts of inorganic matter, the remainder being organic matter soluble in alcohol.

Rate of secretion.—The figures given by various observers for the total quantity of pancreatic juice secreted in twenty-four hours vary greatly, and it is impossible to state an average quantity with any approach to accuracy. Figures obtained from observations on permanent fistulæ greatly exceed those obtained from temporary fistulæ. Bidder and Schmidt place the yield in the dog, at the rate of temporary secretion, at 2·5 grms. per kilo. of body weight per diem. At this rate a man of 70 kilos. (154 lbs.) would secrete 175 grms. of pancreatic juice per diem.

Succus Entericus.

The secretion of the small intestine may be obtained in animals, unmixed with the other digestive secretions, by one of two forms of fistula.

The first form of fistula was introduced by Thiry,[3] and is made by cutting across the intestine at two places, 10 to 30 cms. apart, without interfering with the blood supply, restoring the continuity of the intestine, stitching up one end of the isolated piece, and uniting the other to the wound in the abdominal wall. The second form due to Vella,[4] is a modification in which both ends of the isolated piece of gut are left open and stitched to the abdominal wall one above the other.[5]

Thiry describes the *succus entericus* as a limpid, opalescent, light yellow-coloured fluid, strongly alkaline in reaction, and possessing a specific gravity of 1010.

It contains proteid and mucin, and much carbonate, as shown by effervescence with dilute acids. According to Röhmann,[6] in the dog,

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 160.

[2] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1891, Bd. v. S. 179.

[3] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1864, Bd. l. Abth. 1, S. 77.

[4] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1888, Bd. xiii. S. 40. For details as to establishing such fistulæ, see Gamgee, "Physiological Chemistry," vol. ii. pp. 406–408.

[5] For a full description of the methods of collecting intestinal juice, see article on "Mechanism of Intestinal Secretion."

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. xli. S. 424.

the secretion of the upper part of the small intestine is scanty in quantity, slimy and clot-like, while in the lower part the secretion is much more fluid, and contains small clot-like masses. It contains 4–5 parts per 1000 each of sodium chloride and sodium carbonate.[1] Pregl[2] has recently obtained *succus entericus* from a Vella fistula in the sheep, and estimated its alkalinity as equivalent to 0·454 per cent. of Na_2CO_3. The specific gravity of the fluid averaged 1·014. It contained proteid, and coagulated on standing. Thiry found in the dog, 2·2 to 2·8 of total solids, 0·7 to 1·2 of proteid, 0·7 to 0·9 of ash, per cent.; Leube, 0·8 to 2·7 per cent. of proteid; Quincke, 1·34 to 1·45 per cent. of total solids; Frerichs, 2·27 per cent. of total solids; Gumilewski, 1·5 per cent. of total solids.

Tubby and Manning[3] obtained pure human *succus entericus* from a piece of intestine, $3\frac{1}{2}$ in. in length, situated about 8 in. from the ileo-cæcal valve, for a period of some months; the daily yield from this length of gut averaged 27 c.c. (19 to 35). As a mean of thirty determinations, the specific gravity was found to be 1·0069 (1·0016 to 1·0162). The fluid was generally opalescent, and often had a brownish tint; it contained a few leucocytes and columnar cells, and was free from bacteria. It was invariably alkaline in reaction, and gave off carbonic acid gas on treatment with acids. It gave all the proteid reactions, and did not reduce Fehling's solution or alter the colour of iodine solution. It contained lactates, as shown by darkening a dilute solution of ferric chloride, and giving Uffelmann's test. It also contained much mucin.

Bile.

Action on foodstuffs.—Bile differs from the other digestive secretions in not possessing a marked chemical action on any of the organic foodstuffs. Bile alone is said to exert a diastatic action on starch,[4] but this is very slight and inconstant, and seems to be merely due to a slight absorption of diastatic enzymes;[5] on the other foodstuffs it has no chemical action whatever. Bile also increases the rate of action of pancreatic diastase; but the bile salts alone have a similar effect, so that this accelerating action is not due to a diastatic enzyme.[6]

It has been shown that the presence of bile in the intestine has a favourable influence on the absorption of fat, and that when it is excluded, although the absorption of fat is not stopped, it becomes very defective, and the same amount of fat cannot be taken up as when bile is present. This will be considered later under Fat Absorption.

Chemical composition.—In its physical characteristics and chemical composition the bile is a variable mixture, not only in different classes of animals, but in the same individual. As secreted by the liver cells, and until it reaches the gall bladder, it is a clear limpid fluid, with a low

[1] Gumilewski, *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxix. S. 565.
[2] *Ibid.*, 1896, Bd. lxi. S. 359.
[3] *Guy's Hosp. Rep.*, London, 1891, vol. xlviii. p. 277.
[4] Ewald, "Klinik d. Verdauungskrankheiten," 1890, Bd. i. S. 150.
[5] According to Kaufmann (*Compt. rend. Soc. de. biol.*, Paris, 1890, tome xli. p. 600), the ferment occurs in the bile of the ox, pig, and sheep, in traces in that of the cat, and never in dog's bile. Ellenberg and Hofmeister (*Arch. f. wissensch. u. prakt. Thierh.*, Berlin, 1885, Bd. xi. S. 381, 393) found a diastatic ferment in horse, ox, and sheep bile, and occasionally in that of the dog and pig. In all cases, traces only of ferment are present.
[6] Martin and Williams, *Proc. Roy. Soc. London*, 1889, vol. xlv. p. 358.

percentage of total solids and a correspondingly low specific gravity (1010). In the gall bladder absorption of water takes place,[1] and a mucin-like substance secreted by the epithelium of the gall bladder is added to it, so that it becomes viscid in consistency, the percentage of total solids is much increased, and the specific gravity rises (1030 to 1040).

According to the time it stands in the gall bladder, these changes become more or less advanced, which accounts for much of the variation observed in the quantitative composition of different specimens of bile.

The following table of analyses of dogs' bile, (*a*) from the gall bladder and (*b*) freshly secreted from a fistula, illustrates this difference:[2] —

	In a Hundred Parts by Weight of			
	(*a*) Bile from Gall Bladder.		(*b*) Freshly secreted Bile from a Fistula.	
	I.	II.	I.	II.
Mucin	0·454	0·245	0·053	0·170
Alkaline taurocholates . .	11·959	12·602	3·460	3·402
Cholesterin	0·449	0·133	0·074	0·049
Lecithin	2·692	0·930	0·118	0·121
Fats	2·841	0·083	0·335	0·239
Soaps	3·155	0·104	0·127	0·110
Organic matter insoluble in alcohol . . .	0·973	0·274	0·442	0·543

Bile is an alkaline fluid containing on an average 0·2 per 1000 each of sodium carbonate and alkaline sodium phosphate. It has an intensely bitter taste, leaving a sweetish after-taste in the case of human or ox bile, but not in that of rabbit's or pig's bile. The bile of the ox and some other animals has a faint characteristic odour resembling musk, especially after warming. The colour is very variable: in carnivora it is usually golden-yellow; in herbivora a grass-green; but these colours are not constant, and vary with the amount of oxidation of the bile pigments; the two chief colours are often mixed with brown, giving intermediate shades of yellowish and greenish brown. Human bile, when observed in a healthy condition and immediately after death, is often green, occasionally golden-yellow in colour.

Bile contains no coagulable proteid, and remains clear on boiling; it can also be diluted with water without any turbidity arising. In human bile true mucin is present,[3] but the substance which gives viscidity to ox

[1] Accompanied by a selective absorption of inorganic constituents, so that the percentage of chlorides in gall bladder bile is even less than that in liver bile (Hammarsten, *loc. cit.*, sub. 15).

[2] Hoppe-Seyler, "Lehrbuch der Physiol. Chem.," Berlin, 1881, S. 302. See also Hammarsten, *Nova Acta. Reg. Soc. Sc. Upsala*, 1893, Ser. 3, vol. xvi.; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1893, Bd. xxiii. S. 331.

[3] Hammarsten, *Nova Acta Reg. Soc. Sc.* Upsala, 1893, Ser. 3, vol. xvi.; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1893, Bd. xxiii. S. 333.

bile has been shown not to be mucin but a nucleo-albumin. Other substances present in bile are—(1) the alkaline salts of certain organic acids known as the bile acids; (2) the bile pigments; (3) traces of lecithin, cholesterin, soaps, and fats; (4) mineral salts.

Both the total and relative amount of each of these several constituents or group of constituents is very variable, as is shown by the following table of analyses of human bile made by different observers. The numbers indicate parts by weight contained in 1000 parts by weight of bile:[1]—

	Fresh Bile from Gall Bladder.				Bile from Fistulæ.					
	Frerichs.[2]		v. Gorup-Besanez.[3]		Jacobson.[4]	Yeo and Herroun.[5]	Copeman and Winston.[6]	Mayo Robson.[7]	Nöel Paton and Balfour.[8]	
Water .	860·0	859·2	822·7	898·1	977·4	987·16	985·77	981·98	988·08	984·79
Total solids	140·0	140·8	177·3	101·9	22·6	12·84	14·23	18·02	11·92	15·27
Sodium glycocholate	102·2	91·4	107·9	56·5	10·1	1·65	6·28	7·51	3·56	3·49
Sodium taurocholate						0·55		0·09	0·49	
Cholesterin	1·6	2·6	47·3	30·9	0·56	0·38	0·99	0·45	0·53	0·75
Lecithin .	...	...			0·05			...	...	
Fats . .	3·2	9·2			1·50			0·12	0·09	
Soaps . .	...	...	...	...				0·97	0·15	
Mucin pigment, epithelium, etc. .	26·6	29·8	22·1	14·5	2·3	1·48	1·72	1·30	7·09	4·61
Inorganic salts	6·5	7·7	10·8	6·3	8·5	8·41	4·51	7·58		6·41

The samples of bile from the gall bladder, analysed by Frerichs and by v. Gorup-Besanez, were obtained immediately after death from healthy subjects, the others were from biliary fistulæ of long standing.

Specific Constituents of Bile.

Nucleo-proteid of bile.—Landwehr[9] first drew attention to the fact that the percentage composition of the mucin of bile was different from that of other mucins, and that no reducing sugar was formed on heating it with a mineral acid, but attempted to explain this by assuming that

[1] Extracted partially from Bunge, "Lehrbuch der physiol. und pathol. Chemie," Leipzig, 1894, S. 192; and partially from Nöel Paton and Balfour, *Rep. Lab. Roy. Coll. Phys.*, Edin., 1891, vol. iii. p. 191.

[2] *Hannover. Ann. f. d. ges. Heilk.*, 1845, N.F. Bd. v. S. 42.

[3] *Prager. Vrtljschr. f. prakt. Pharmakol.*, 1851, Bd. iii. S. 86.

[4] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1873, Bd. vi. S. 1026.

[5] *Journ. Physiol.*, Cambridge and London, 1884, vol. v. p. 116.

[6] *Ibid.*, 1889, vol. x. p. 213.

[7] *Proc. Roy. Soc. London*, 1890, vol. xlvii. p. 499.

[8] *Loc. cit.*

[9] *Ztschr. f. physiol. Chem.*, Strassburg, 1881, Bd. v. S. 371.

a glycogen-like substance was present in the other mucins, which, on boiling with a mineral acid, formed a reducing sugar ; this substance he supposed to be absent in bile mucin, and hence no reducing sugar was formed on heating it with a dilute mineral acid.

Paijkull[1] afterwards proved that the mucin-like substance which gives bile its viscidity really belongs to the nucleo-proteids. If bile be precipitated with dilute acetic acid, the presence of the bile salts prevents the precipitate from redissolving in excess, and so causes it to simulate a mucin ; but if the bile salts are removed by dialysing, or by precipitating the substance with alcohol, centrifugalising and quickly redissolving in water, the precipitate readily redissolves in excess of acetic acid, and in this respect resembles a nucleo-proteid and not a mucin. Also, when the substance is precipitated by, and just redissolved in, dilute hydrochloric acid, and then subjected to peptic digestion, a substance is precipitated, which by its percentage of phosphorus can be recognised as similar to the nuclein yielded under like conditions by nucleo-proteids. These facts, together with the much higher percentage of nitrogen (14 to 16 per cent.) than mucin which it contains, and its failing to yield a reducing sugar on boiling with dilute mineral acids, show the substance to be a nucleo-proteid and not a mucin. The quantity of this substance present in bile is very variable but always small, amounting in ox bile to about one per thousand.[2]

The bile salts.—There are found in bile the salts of a number of organic acids of complicated structure, which are closely allied to one another ; these salts are collectively called the bile salts. They are not found in health in appreciable quantity elsewhere than in the bile, and usually occur as sodium salts, except in the bile of some sea fishes, in which they are present as potassium salts.

Since bile is so easily obtainable in quantity, it is not surprising that it should early have attracted the notice of the physiological chemist. Thénard, in 1809, working with ox bile, was the first to obtain any scientific knowledge of the bile acids. He distinguished two components in bile, one precipitated by acetate of lead, which he called *bile resin*, and a soluble part, which he named *picromel*. He seems to have roughly separated in an impure condition those two most commonly occurring bile acids, which we know to-day as glycocholic and taurocholic acid, by a method not widely differing from that most used at the present time. He precipitated bile with neutral and basic acetates of lead, and then extracted the precipitate with nitric acid ; the insoluble part left behind, his *resin of bile*, was impure glycocholic acid. The filtrate he reprecipitated with excess of acetate of lead, collected the precipitate, and decomposed it by a current of sulphuretted hydrogen, thus obtaining his *picromel*, which must have corresponded to impure taurocholic acid.

In 1826, Gmelin published a memoir[3] in which is described a large number of bile constituents ; amongst them, one corresponding to glycocholic acid, which he obtained in a crystalline form ; and another substance, taurine, of which he was the discoverer, although he wrongly supposed that it existed ready formed in the bile. The next important advance was made by Demarcay,[4] who obtained a substance (*Choleinsäure*) which yielded, on heating with acids, taurine and a resinous substance,

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 196. [2] Paijkull, *loc. cit.*
[3] "Die Verdauung nach Versuchen."
[4] *Ann. d. Chem.*, Leipzig, 1838, Bd. xxvii. S. 270.

and, on treatment with alkalies, ammonia and a non-nitrogenous acid, corresponding to what to-day is called cholalic acid.

In 1844, Plattner[1] succeeded in obtaining the bile salts in a crystalline form, and so laid a sure foundation for all succeeding work on the isolation and study of the bile acids. He also showed, by boiling this crystalline product with acid, that taurin is a decomposition product and does not exist as such in bile. Redtenbacher[2] previously to this had shown that this body contains sulphur, and established its formula as $C_2H_7NSO_3$. Plattner[3] afterwards discovered a simpler method of obtaining the mixed bile salts in crystalline form. He concentrated the bile without decolorising, and then added an excess of alcohol, warmed, and after some time filtered and added ether, till a brown sticky precipitate began to fall; this was allowed to settle, and the clear fluid decanted off, cooled, and treated with more ether from time to time. The bile salts alone being the only constituents which are soluble in water and alcohol, and insoluble in ether, are slowly thrown out of solution; and on standing for some days or weeks in the cold, under the alcoholic ethereal mother-liquid, form themselves into ball-shaped masses, or starlike clusters of fine needles, which increase in size on standing. This crystalline mass is known as "*Plattner's crystallised bile.*" The crystals are dried between filter-paper, washed with alcohol, containing 1 in 10 of ether, purified by recrystallisation, and dried over sulphuric acid.

This discovery of Plattner's paved the way for the classical researches of Strecker, to whom we owe the greater part of any exact knowledge we have of the bile acids. Strecker[4] first showed that "*Plattner's crystallised bile*" consists of a mixture of the sodium salts of two acids, which are so related to each other that they yield, on boiling with acids, a common non-nitrogenous constituent, cholalic acid, and a nitrogenous constituent, which in both cases is an amido-acid. One of these amido-acids is glycocoll or amidoacetic-acid, the other taurine or amidoethylsulphonic-acid. Of the two bile acids the one which yields glycocoll and cholalic acid is called glycocholic acid, while the other, which yields taurine and cholalic acid, is named taurocholic acid.

Cholic or cholalic acid is not, however, the only basis of the different varieties of bile acids; other acids closely allied to it in percentage composition, but quite distinct from it, have been isolated. In ox bile about a third part of the cholalic acid is replaced by an acid called choleic acid.[5] In human bile an acid called fellic acid[6] has been described as occurring along with cholalic and choleic acids; and modified cholalic acids are present in the hyoglycocholic acid of pig's bile and the chenotaurocholic acid of goose bile. None of these substitutes of cholalic acid occur free in bile, but always combined with glycocoll or taurine to form modified glycocholic or taurocholic acids; they are all soluble with difficulty in water and ether, and easily soluble in alcohol.[7]

[1] *Ann. d. Chem.*, Leipzig, 1844, Bd. li. S. 105. [2] *Ibid.*, 1846, Bd. lvii. S. 170.

[3] *Journ. f. prakt. Chem.*, Leipzig, 1847, Bd. xi. S. 129.

[4] *Ann. d. Chem.*, Leipzig, 1848, Bd. lxv. S. 1; 1848, Bd. lxvii. S. 1; 1849, Bd. lxx. S. 149.

[5] Latschinoff, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1885, Bd. xviii. S. 3039; 1886, Bd. xix. S. 1140; 1887, Bd. xx. S. 1043.

[6] *Fellinsäure* of Schotten, *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 268. See also Lassar-Cohn, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1894, Bd. xxvii. S. 1339.

[7] Hammarsten's "Lehrbuch," 1895, S. 198. He describes a third variety of bile acid, found in shark's bile, which is rich in sulphur, and from which boiling with hydrochloric acid splits off sulphuric acid.

The alkaline salts of the bile acids are soluble in water and alcohol, but insoluble in ether, and these solubilities form the basis of Plattner's method of separating them from the other biliary constituents. This is best done by mixing the bile with freshly-heated animal charcoal, evaporating to complete dryness, and then extracting with absolute alcohol, which takes up the bile salts along with cholesterin and traces of lecithin, fats, and soaps; but, on addition of excess of ether, only the bile salts are thrown out of solution.

The relative amount of each of the bile acids present in bile varies within wide limits. In the bile of carnivora, glycocholate of sodium is present in very small quantity; for example, the bile salts of dog's bile consist exclusively of taurocholate of sodium,[1] while in most herbivora the glycocholate is usually present in greater quantity than the taurocholate; to this rule the goat and sheep are said to be exceptions.

In human bile most of the cholalic acid is combined with glycocoll, occasionally the whole of it.[2] Hammarsten's[3] analysis of the mixed bile salts of healthy human bile gave 13·1 per cent. taurocholic acid, 86·9 per cent. glycocholic acid. Since glycocholic acid is sulphur-free, and the percentage in taurocholic acid is known, the relative amount of the two acids may be determined from the percentage of sulphur in a preparation of *Plattner's crystallised bile*, obtained from any given sample of bile.

The isolation of each of the bile acids from a mixture of their salts is usually a lengthy and difficult process, especially in the case of taurocholic acid, which can only with great difficulty be freed from glycocholic acid, so that taurocholic acid is usually prepared from dog's bile, while glycocholic acid is prepared from ox bile.

Both free acids behave like their sodium salts in being soluble in alcohol and insoluble in ether, but differ in that taurocholic acid is easily soluble in water, while glycocholic acid is soluble with great difficulty. On this property is based the simplest method of obtaining pure glycocholic acid, that of Hüfner;[4] unfortunately, the presence of taurocholic acid confers solubility on the glycocholic acid, so that the method often fails when too much taurocholate is present in the sample of bile experimented upon.

The method consists in adding to fresh ox bile a few drops of hydrochloric acid, and filtering from the precipitated pseudo-mucin. To 100 c.c. of this filtrate 5 c.c. of concentrated hydrochloric acid and 30 c.c. of ether are added. The hydrochloric acid sets free both bile acids, and the glycocholic acid is precipitated in crystalline form (unless too much taurocholic acid be present), either immediately, or on standing some hours in the cold. The ether added aids in the production of this crystalline precipitate, which is next washed with acidulated water saturated with ether, and finally recrystallised from boiling water.

Marshall[5] tested Hüfner's method with 543 samples of ox bile, and obtained a precipitation in 121 cases. A similar method was employed by Strecker,[6] using a watery solution of crystallised bile instead of fresh bile.

[1] Strecker, *Ann. d. Chem.*, Leipzig, 1849, Bd. lxx. S. 178; Hoppe-Seyler, *Journ. f. prakt. Chem.*, Leipzig, 1863, Bd. lxxxix. S. 283.

[2] Jacobson, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1873, Bd. vi. S. 1028.

[3] *Schmidt's Jahrb.*, Leipzig, 1879, Bd. clxxxi. S. 5.

[4] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, Bd. iv. S. 301.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 233.

[6] *Ann. d. Chem.*, Leipzig, 1848, Bd. lxv. S. 1.

The different solubilities of the lead salts of the two acids provides another means of separating glycocholic acid; the separation of pure taurocholic acid from the mixture by this method is more difficult.

Glycocholate of lead is thrown out of solution on the addition of neutral acetate of lead to a solution of a mixture of the bile salts; the remainder of the glycocholate and all the taurocholate are thrown down on the addition of ammonia or of basic acetate of lead to the filtrate.

Fresh ox bile is treated with alcohol to precipitate the pseudo-mucin. The alcohol is evaporated off, and neutral acetate of lead added as long as a precipitate forms; this precipitate is collected and decomposed by warming with a solution of sodium carbonate, whereby sodium glycocholate is formed; the mixture is next evaporated to dryness, and extracted with alcohol, in which the sodium glycocholate dissolves. This alcoholic solution is filtered, the filtrate is evaporated to dryness, and the residue is dissolved in water. The watery solution of sodium glycocholate so obtained is decolorised with animal charcoal, and the glycocholic acid thrown out of solution by adding a mineral acid. Finally, it can be recrystallised, either from boiling water, or by the addition of ether to its alcoholic solution. Taurocholic acid can be obtained from the filtrate from neutral acetate of lead, by fractional precipitation with basic acetate of lead, as the remaining glycocholate unprecipitated by the neutral acetate is precipitated by the portion of basic acetate first added.[1] Basic acetate of lead is stirred into the filtrate from the neutral acetate, until the precipitate commences to gather into a sticky mass, when the addition is discontinued, and the solution decanted off from the precipitate. More basic acetate solution is now added, and throws down a plastic mass, consisting of fairly pure taurocholate of lead. This precipitate is dissolved in boiling alcohol, filtered warm into water, and the resulting reprecipitated mass, after being purified by kneading, is dried, dissolved in a small quantity of alcohol, decomposed with sulphuretted hydrogen, filtered from lead sulphide, and dried at first in the air, afterwards in a vacuum over sulphuric acid.

Taurocholic acid is, however, best prepared from dog's bile, as described by Parke.[2]

The bile is evaporated down, extracted with alcohol, decolorised with animal charcoal, evaporated to dryness, dissolved in absolute alcohol, and treated with excess of ether. After some time the crystalline precipitate of sodium taurocholate so obtained is dissolved in water, and the solutions precipitated with acetate of lead and ammonia. The precipitate is collected, washed, suspended in alcohol, or dissolved therein by boiling, and decomposed by sulphuretted hydrogen. The filtrate from sulphide of lead is evaporated to a small volume, and mixed with excess of ether, when the taurocholic acid is precipitated as a syrup, in which, after some time, small crystals appear. These are in the form of fine needles which deliquesce in the air.

Glycocholic acid ($C_{26}H_{43}NO_6$) is a monobasic acid, crystallising in long fine needles, which fell together into a light, voluminous mass when first formed from a solution, and on drying form a loose, snowy white mass with a silky glance. These crystals melt at 100° C., losing water in so doing and forming glycocholonic acid; they are very sparingly soluble in cold water (1 in 300), somewhat more soluble in boiling water (1 in 120), and so can easily be recrystallised from hot water; they are easily soluble in alcohol and in acetic acid, but soluble in ether with great difficulty. Glycocholic acid and its salts in solution

[1] Lieberkühn, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1852, Bd. i. S. 113.
[2] *Hoppe-Seyler's Med.-chem. Untersuch.*, Berlin, S. 160.

rotate the plane of polarised light to the right; in alcoholic solution the specific rotatory power for the acid is +20°·0, for the sodium salt +25°·7 (Hoppe-Seyler). The salts of the alkalies and alkaline earths are soluble both in water and in alcohol, those of the heavy metals are mostly much more insoluble in water, so that addition of salts of such metals as lead, copper, iron, or silver, causes precipitation of the corresponding glycocholates. The lead salt is soluble in rectified spirit, from which it is precipitated on the addition of water. The acid and its salts possess a peculiar taste, sweetish at first, but afterwards intensely bitter.

Taurocholic acid ($C_{26}H_{45}NSO_7$), also a monobasic acid, is crystallisable with difficulty, forming fine deliquescent needles. It is very easily soluble in water, and also possesses the power of carrying glycocholic acid into solution when that acid is simultaneously present. It is exceedingly soluble in alcohol, but insoluble in ether. In solution it possesses a bitter-sweet taste, which is shared by its alkaline salts. The salts are generally easily soluble in water, and a solution of an alkaline taurocholate, unlike that of a glycocholate, is not precipitated by the usual salts of the heavy metals, such as copper sulphate, silver nitrate, or neutral lead acetate; basic lead acetate does, however, precipitate it, and the compound so formed is soluble in boiling alcohol.

Taurocholic acid is not nearly so stable a compound as glycocholic acid, it decomposes on boiling in aqueous solution, or in evaporating to dryness; hence the dry pure acid has never been prepared or analysed, and its formula has been deduced from analogy with glycocholic acid, and from analyses of its more stable salts. Its solutions rotate the plane of polarisation to the right, like glycocholic acid. The specific rotation of the alcoholic solution of the sodium salt is +24°·5. Potassium taurocholate occurs in the bile of many fishes; it possesses the peculiar property of being completely thrown out of solution in water by the addition of solution of caustic potash, and so may be prepared by adding this reagent to an aqueous solution of an alkaline taurocholate. Analyses of this salt by Strecker[1] established its formula as $C_{26}H_{44}KNSO_7$, and analyses of the sodium salt gave a corresponding result, from which it follows that the formula of taurocholic acid itself is $C_{26}H_{45}NSO_7$.

Hyoglycocholic acid is an acid found in pig's bile,[2] which yields on decomposition glycocoll, like ordinary glycocholic acid, but an acid differing in composition and behaviour from ordinary cholalic acid ($C_{24}H_{40}O_5$), and called hyocholalic acid ($C_{25}H_{40}O_4$). This acid differs from cholalic acid in not being so easily crystallisable, and in having a difficultly soluble barium salt. Severin Jolin[3] states that pig's bile contains, as principal bile salts, the sodium salts of two different hyoglycocholic acids, each of which yields on decomposition glycocoll and a hyocholalic acid (α and β). The two hyoglycocholic acids are distinguished by the different solubilities of their sodium salts in neutral salt solutions. The β-salt is present in much greater quantity; but the distinguishing character of pig's bile, that it is precipitated by saturation with various neutral salts, is not due to the β- but to the α-hyoglycocholic acid.

[1] *Loc. cit.*

[2] Strecker and Grundelach, *Ann. d. Chem.*, Leipzig, 1847–9, Bd. lxii. S. 205; Bd. lxx. S. 179.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1887-9, Bd. xi. S. 417; Bd. xii. S. 512; Bd. xiii. S. 205.

The two hyocholalic acids show analogous differences to the two hyoglycocholic acids. The formula of α-hyoglycocholic acid is $C_{27}H_{43}NO_5$, that of β-hyoglycocholic acid is $C_{26}H_{43}NO_5$.

Taurochenocholic acid,[1] the principal bile acid of goose bile, has the formula $C_{29}H_{49}NSO_6$, has not been crystallised, and is soluble in water and alcohol. From this acid Heintz and Wislicenus[2] prepared chenocholic acid ($C_{27}H_{44}O_4$); this is itself crystallisable with difficulty, but yields a barium salt, which is insoluble in water and can easily be obtained in a crystalline form.

Pettenkofer's test for bile acids.[3]—When bile is gently warmed with concentrated sulphuric acid and cane-sugar, a beautiful purple or purplish-red colour develops, becoming deeper on standing. The colour is due to an interaction between the bile salts, or cholalic acid, and a substance called furfurol or furfuraldehyde developed by the action of the strong sulphuric acid on the cane-sugar;[4] hence the test may be more satisfactorily carried out where only traces of bile salts are suspected, by using a solution of furfurol (1 per 1000) instead of cane-sugar.

To carry out the test in the ordinary manner, add to a drop or two of the bile, or fluid suspected of containing bile acids, a drop of strong sulphuric acid, taking care that any great rise in temperature does not occur; spread the mixture out in a thin film in a porcelain capsule, and either add a drop of a 10 per cent. solution or a small crystal of cane-sugar; if the violet colour does not appear at once, warm very gently. To carry out the test with furfurol, one drop of a solution of furfurol (1 per 1000) is added to 1 c.c. of an alcoholic solution of bile salts, and 1 c.c. of concentrated sulphuric acid is added cautiously to this, so as not to overheat. In this manner $\frac{1}{20}$–$\frac{1}{30}$ of a milligramme of cholalic acid may be detected.[5]

The test with sugar may be easily spoiled by overheating or when too much sugar is used, which favours carbonisation. The presence of sulphurous acids or nitrous fumes in the sulphuric acid is also unfavourable to the reaction. Strong phosphoric acid may be used instead of sulphuric acid.

Many other substances give a similar reaction. Pettenkofer himself was aware that proteids gave a similar colour, though much less easily. By subsequent observers[6] a large number of substances giving colour reactions with furfurol have been described; amongst these many phenols and aromatic bases are included, some of which are also found in the urine. v. Udránszky[7] gives a list of over forty substances which give colour reactions with furfurol, but none except α-naphthol show the reaction with the same delicacy as the bile salts. That the coloured substance so produced is not in all cases the same, is shown by the fact that some possess no absorption spectrum, and that the spectra of the others differ from one another. In this way the spectrum of the colour given by the bile salts may be distinguished

[1] Marsson, *Arch. d. Pharm.*, Bd. lvii. S. 138.
[2] *Ann. d. Phys. u. Chem.*, Leipzig, 1859, Bd. cviii. S. 547.
[3] *Ann. d. Chem.*, Leipzig, 1844, Bd. lii. S. 90.
[4] Mylius, *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 492.
[5] v. Udránszky, *ibid.*, 1888, Bd. xii. S. 355.
[6] Baeyer, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1872, Bd. v. S. 26; Stenhouse, *Ann. d. Chem.*, Leipzig, 1870, Bd. clvi. S. 197; Schiff, *ibid.*, Bd. cci. S. 355.
[7] *Loc. cit.* Drechsel, *Journ. f. prakt. Chem.*, Leipzig, Bd. xxvii. S. 424.

from the others by two bands, one between the solar lines D and E near to E, the other at F.[1]

The bile salts produce great slowing of the heart's beat, which may be used as a physiological test for them in confirmation of Pettenkofer's reaction. In a curarised frog the heart is exposed, the pericardium removed, and the action of the vagus paralysed by atropine; on now adding a drop of a solution of a bile salt, the rhythm of the heart is greatly slowed.[2]

Cleavage products of the bile acids.—All the bile acids, under the action of hydrating agents, split up into two components, of which one is always either glycocoll or taurin, and the other a non-nitrogenous monobasic acid which may be cholalic acid or one of several allied acids.

Glycocoll and taurin are nitrogenous bodies, belonging to that class of substances called amido-acids, *i.e.* organic acids, in which one or more hydrogen atoms are replaced by the group amidogen (NH_2). Both these amido-acids are probably formed by the breaking up of proteids, or their allies the albuminoids.

The process of hydration can be carried out directly from bile, by heating with hydrochloric acid, in a flask attached to a reversed condenser. Taurin and hydrochlorate of glycocoll are formed, and the free cholalic acids, which slowly lose water and pass into the form of their anhydrides (the dyslysins, p. 382); these being insoluble are precipitated. As soon as the reaction is completed, as shown by the failure of Pettenkofer's test, the flask is allowed to cool and the dyslysins filtered off. The filtrate, which contains the amido-acids, is strongly concentrated, and, while still warm, decanted from the sodium chloride which has crystallised out. It is next evaporated to complete dryness and treated with absolute alcohol, which takes up the glycocoll hydrochlorate and leaves the taurin behind. The residue is dissolved in as small a quantity as possible of warm water, and filtered while warm; to this filtrate a little alcohol is added, and, on slowly cooling, crystals of taurin are formed.

The alcohol is evaporated from the alcoholic extract containing the glycocoll hydrochlorate, and water is added; to the watery solution, hydrate of lead is added, when insoluble lead chloride and a soluble lead compound of glycocoll are formed. The latter is separated in solution by filtration; into the solution a stream of sulphuretted hydrogen is passed, the lead sulphide is filtered off, and the filtrate is concentrated, until, on cooling, free glycocoll crystallises out.

The free cholalic acids[3] can be recovered from the dyslysins formed in the first step of the above process. The dyslysins are removed from the filter, and boiled with dilute alkali, when they take up water, and, combining with some of the alkali, are converted into soluble alkaline cholalates. On acidifying with hydrochloric acid, and evaporating to dryness, the cholalic acids can be extracted with a small quantity of hot alcohol, from which they crystallise on cooling, or on the addition of excess of ether.

Glycocoll, glycocine, or glycine, is amido-acetic acid ($NH_2.CH^2.COOH$). Besides occurring combined with cholalic acid, as glycocholic acid in the bile, it is found in the urine of certain animals and occasionally in man, combined with benzoic acid, to form hippuric acid, and is formed as an end hydration product from gelatine and similar substances.

[1] Koschlakoff and Bogomoloff, *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, Bd. vi. S. 529. In this paper four bands are described. Bogomoloff, *ibid.*, 1869, Bd. vii. S. 529; Schenck, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 232.

[2] Mackay, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1885, Bd. xix. S. 279.

[3] The term "cholalic acids" is used to signify cholalic acid and its allies.

It crystallises in colourless rhombohedra, or in four-sided prisms, which have a sweet taste and dissolve easily in cold water (1 in 4·3); in alcohol and ether they are insoluble. Glycocoll, like other amido-acids, can act chemically, either as a base or an acid in forming compounds with acids and bases respectively. As a type of these combinations with bases, the copper compound may be taken. When freshly precipitated cupric hydrate is added to a warm concentrated solution of glycocoll, it dissolves to form a deep blue solution, which is not reduced on boiling; on cooling this solution, or on adding alcohol and allowing to stand, fine dark blue needles crystallise out of the composition $(NH_2.CH_2.CO_2)_2Cu,H_2O$. Glycocoll has been obtained synthetically by the action of ammonia on monochloracetic acid thus:—

$$NH_3 + CH_2.Cl—COOH = CH_2.(NH_2)—COOH + HCl.$$

Taurine is amido-isethionic acid, also called amido-oxyethylsulphonic acid ($NH_2.C_2H_4.SO_2OH$).[1] It occurs in the body, apart from the bile, only in minute and inconstant traces; it has been stated to occur in the lungs and kidneys of oxen, in some of the organs of cold-blooded animals, and in inconstant traces, probably due to the decomposition of taurocholates, in the intestine. The presence of sulphur in its molecule shows it to be formed from proteids in the body; but the intermediate steps in its formation are unknown.

Taurine is very easily crystallised, and forms large colourless prismatic prisms with a glassy glance,[2] without any taste, and gritty between the teeth, neutral in reaction and very stable, not being altered by a temperature of 240 °C.; heated above this temperature they melt and decompose in so doing. It is much less soluble in cold water than glycocoll, but still easily soluble (1 in 15·5), and still more so in hot water; in alcohol and ether it is insoluble. It is soluble in concentrated sulphuric and nitric acids without decomposition, and the latter acid may even be boiled off, leaving it unaffected; neither is it affected by boiling with aqua regia. To alkalies also it is much more stable than glycocoll; it is not affected by weak alkalies, and only by continued boiling in strong alkaline solution is slowly broken up into ammonia, acetic, and sulphurous acids; so that it is one of the most stable of the organic compounds found in the body. Taurine is also a much more neutral substance in its chemical behaviour than glycocoll; it does not combine at all with acids, and its affinity for bases is very feeble. An amorphous mercury compound is however obtained by boiling a solution of taurine with freshly-precipitated mercuric oxide.[3]

The constitution of taurine is shown by its synthesis from chlorethylsulphonic acid by the action of ammonia.[4]

$$SO_2\begin{matrix}C_2H_4Cl\\OH\end{matrix} + {}_2NH_3 = SO_2\begin{matrix}C_2H_4NH_2\\OH\end{matrix} + NH_4Cl$$

This synthesis, as well as the fact that taurine is not saponified by dilute alkalies, shows that taurine is not an ester but a sulphonic derivative; that is, that the sulphur atom is united directly to carbon, and not indirectly by oxygen. Taurine may also be obtained by heating

[1] The amido-oxyethyl radicle is directly united to sulphur in the molecule.
[2] Gmelin, "Verdauung nach Versuchen," S. 60.
[3] Lang, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, Bd. vi. S. 74.
[4] Kolbe, *Ann. d. Chem.*, Leipzig, 1862, Bd. cxxii. S. 33.

the ammonium salt of oxyethylsulphonic acid to 230° C., when a molecular rearrangement takes place, thus:

$$SO_2\begin{matrix}\diagup C_2H_4.OH \\ \diagdown ONH_4\end{matrix} = SO_2\begin{matrix}\diagup C_2H_4.NH_2 \\ \diagdown OH\end{matrix} + H_2O$$

Cholalic acid is the usual partner with glycocoll or taurine in the formation of the bile acids; it is also found in the intestinal contents, and sometimes, in cases of jaundice, in the urine. One method of obtaining it has already been incidentally mentioned, but it is better prepared by the following method:[1]—

Ox bile is boiled for about twenty-four hours with the fifth part of its volume of 30 per cent. caustic soda solution, the water being replaced as it is removed by evaporation. The solution is then saturated with carbon-dioxide gas, and evaporated almost to dryness. The residue is extracted with 96 per cent. alcohol, and the extract, diluted so that it does not contain above 20 per cent. of alcohol, is completely precipitated with a solution of barium chloride. The precipitate, which consists of impure barium choleate, is filtered off, and cholalic acid is precipitated from the filtrate by the addition of hydrochloric acid. The acid slowly becomes crystalline on standing, when it is separated and purified by repeated recrystallisation from alcohol. Cholalic acid occurs in many crystalline forms.[2] Anhydrous crystals forming flat-ended 4- to 6-sided prisms, may be obtained by dissolving the amorphous form of the acid, produced by drying one of the other crystalline forms, in ether and allowing the solution to crystallise out.

From strong alcohol the acid crystallises, on the addition of a very little water, in octohedra and tetrahedra, belonging to the orthorhombic system, and containing two and a half molecules of water of crystallisation; from dilute alcohol it crystallises in fine shining flat needles or plates, containing only one molecule of water of crystallisation.[3] It also crystallises in large rhombic tetrahedra, or octohedra containing one molecule of alcohol. Pure anhydrous cholalic acid melts at 194° to 195° to a colourless liquid; and on heating above this temperature, loses water and is converted into its anhydride or dyslysin; on further heating, it loses more water and yields a viscid yellow or yellow-brown oil with a green fluorescence; this is another anhydride, with the composition $C_{48}H_{66}O_3$.

All forms of the acid are sparingly soluble in water and ether, and easily soluble in alcohol. The solutions possess the bitter-sweet taste of bile. The alkaline salts are crystalline and soluble in water, but precipitated by strong solutions of alkalies or their carbonates. The barium salt is much more soluble in cold water (1–30) than the corresponding salts of the allied acids described below. Cholalic acid and its soluble salts turn the plane of polarisation to the right.[4] Methyl and ethyl ethers of cholalic acid have been obtained.

The formula of cholalic acid was first established by Strecker[5] as $C_{24}H_{40}O_5$, and this formula, after some dissent,[6] is now generally accepted. With regard to its constitution, in spite of a vast amount of labour on the subject, we are still only possessed of very fragmentary and uncertain information. It is certainly a monobasic acid, and must

[1] Mylius, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 262.
[2] See Maly, Hermann's "Handbuch," Bd. v. (2), S. 136.
[3] Schotten, *Ztschr. f. physiol. Chem.*, Strassburg, 1886–7, Bd. x. S. 175; xi. S. 268.
[4] Hoppe-Seyler, *Journ. f. prakt. Chem.*, Leipzig, 1863, Bd. lxxxix. S. 265; E. Vahlan, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 253.
[5] *Loc. cit.*
[6] Latschinoff, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1887, Bd. xx. S. 1968.

therefore contain one carboxyl group (COOH), and according to Mylius[1] it also contains one secondary (CHOH) and two primary alcohol groups (CH_2OH).

The evidence for this is derived from its behaviour on cautious oxidation. It first yields, when oxidised, monobasic dehydrocholalic acid ($C_{24}H_{34}O_5$),[2] and on further oxidation tribasic bilic, or bilianic acid ($C_{24}H_{34}O_8$).[3] These changes may be expressed by supposing that, in the formation of dehydrocholalic acid, the two primary alcohol groups form aldehyde groups (H—C = O), and the secondary group, a ketone group (C = O), and that in the further formation of bilianic acid the two aldehyde groups pass into (acid or) carboxyl groups, so producing a tribasic acid; while besides, in the rest of the molecule, an additional ketone group is formed, as shown by the following formulæ:—

Cholalic acid, $C_{20}H_{31}(CHOH)(CH_2OH)_2(COOH)$, on oxidation forms, in place of one secondary alcohol group (CHOH) and two primary alcohol groups (CH_2OH), one ketone group (CO) and two aldehyde groups (COH), thus yielding dehydrocholalic acid, $C_{20}H_{31}(CO)(COH)_2(COOH)$, in which, on further oxidation, an additional ketone group is formed, and the aldehyde groups change into carboxyl groups (COOH), thus yielding the tribasic acid, bilianic (or bilic) acid, $C_{19}H_{31}(CO.)_2(COOH)_3$.

Scarcely anything is known of the arrangement of the atoms in the hydrocarbon part of the molecule. Mylius[4] has obtained a reaction between cholalic acid and iodine, in solution, with the formation of a blue compound, which is crystallisable and becomes easily dissociated in the same manner as iodide of starch. For example, in solution, it becomes decolorised on heating.

This substance is probably an addition product of cholalic acid and iodine, and so points out that the hydrocarbon radicle of the acid is not fully saturated; beyond this, however, we know nothing of its composition.

Desoxycholalic acid is a reduction compound obtained by Mylius[5] of the formula $C_{24}H_{40}O_4$.

Choleic acid[6] was first found in the preparation of cholalic acid from ox bile, and separated from it by means of the more sparing solubility of its barium salt. According to Lassar-Cohn, it also occurs in human bile, and its formula is $C_{24}H_{40}O_4$. Latschinoff,[7] its discoverer, ascribed to it the formula $C_{25}H_{42}O_4$. From Lassar-Cohn's[8] formula it appears to be isomeric, or perhaps identical, with desoxycholalic acid.

Fellic acid[9] ($C_{23}H_{40}O_4$) is an acid which has been obtained from human bile; it is crystalline, insoluble in water, and forms insoluble barium and magnesium salts.

The acids formed by the cleavage of the peculiar bile acids found in the bile of the pig and goose have been already mentioned in treating of these acids.

[1] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1886, Bd. xix. S. 369, 2000.

[2] Hammarsten, *ibid.*, 1881, Bd. xiv. S. 71 (*Dehydrocholsaüre*); Lassar-Cohn, *ibid.*, 1892, Bd. xxv. S. 805; *Ztschr. f. physiol. Chem.*, Strassburg, 1892, Bd. xvi. S. 488.

[3] Cleve, *Bull. Soc. chim.*, Paris, tome xxxv.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 306; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1887, Bd. xx. S. 683.

[5] *Loc. cit.*

[6] *Choleinsäure* of Latschinoff, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1885, Bd. xviii. S. 3039.

[7] *Loc. cit.*

[8] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1894, Bd. xxvii. S. 1339.

[9] *Fellinsäure* of Schotten, *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 268. See also Lassar-Cohn, *ibid.*, 1894, Bd. xix. S. 563.

The acids formed as cleavage products from human bile are cholalic, choleic, and fellic acids.

Cholalic acid and its allies, on boiling with acids, on heating in the dry state, or by putrefaction, lose water, and become converted into anhydrides, or, as they are called, dyslysins. The dyslysin corresponding to cholalic acid has the formula $C_{24}H_{36}O_3$; it is found in fæces; is a white amorphous substance, insoluble in water and alcohol, soluble in ether and melting at 140° C. Another compound, choloidinic acid, is formed, as an intermediate stage, of the formula $C_{24}H_{38}O_4$. On boiling with alkalies, dyslysin takes up water, and is reconverted into cholalic acid.

The bile pigments and their derivatives.—The variations in the colour of bile early attracted attention, and Gmelin,[1] in 1826, first obtained proof of a relationship between these colours, and described the test which still bears his name. He was aware that the play of colours was due to a process of oxidation, and made an experiment to illustrate this by acidifying bile with hydrochloric acid, and enclosing it in a tube from which the air was shut off by a mercury trap. Under these circumstances no change in colour took place; but on exposing the acidified bile to the air, a green colour slowly developed. He also accurately described the play of colours obtained on oxidising with nitric acid.

Berzelius[2] precipitated biliverdin from ox bile with barium chloride, purified it to some extent, and described its properties, but he fell into the error of supposing that it was identical with chlorophyll.[3]

Heintz,[4] preventing oxidation by exclusion of air, extracted from gallstones a brown amorphous pigment, which he named biliphäin. He analysed it, and converted it by dissolving in sodium carbonate, and leading oxygen through the solution into a green pigment, biliverdin. His biliphäin corresponded to the bilirubin of the present day, and his experiment shows well the connection between the two pigments.

Valentiner,[5] in 1859, was the first to obtain bilirubin in a crystalline form, by dissolving in chloroform, from which, on evaporation of the solvent, it crystallises in microscopic crystals. From this discovery onwards, research on the bile pigments took a more exact form, as methods for the isolation of the pigments were discovered and perfected.[6]

Although a considerable number of more or less well-characterised bile pigments have been described, only two are found under normal conditions in the bile, these are bilirubin and biliverdin; the others are obtained by artificial means from these, are found under pathological conditions only in the body, or are formed after death. The colour of the bile is a compound of the colour of these two pigments, and varies with the varying ratio of their amounts through all shades between

[1] Tiedemann and Gmelin, "Verdauung nach Versuchen," 1826.

[2] "Chemie," S. 281.

[3] The spectra of phylloporphyrin and hæmatoporphyrin and their derivatives are almost identical, and in other respects the substances closely resemble each other, so that there is undoubtedly a relationship between them (Schunck and Marchlawski, *Proc. Roy. Soc. London*, Jan. 1896). Now, phylloporphyrin is a derivative of chlorophyll, and hæmatoporphyrin is isomeric with bilirubin, so that there may be some remote connection between biliverdin and chlorophyll.

[4] *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1851, Bd. ii. S. 59; *Ann. d. Phys. u. Chem.*, Leipzig, 1851, Bd. lxxxiv. S. 106.

[5] *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1859, Bd. ii. S. 87.

[6] Brücke, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, Bd. vi. S. 173; Städeler, *Vrtljschr. d. naturf. Gesellsch. in Zurich*, 1863, Bd. viii. S. 1; *Ann. d. Chem.*, Leipzig 1864, Bd. cxxxii. S. 323.

reddish-brown and grass-green. To the variation in relative amount of these two pigments is also due the difference in colour between fresh and stale bile. When bile stands in the gall bladder, its pigments become reduced, the biliverdin is converted into bilirubin, and the colour becomes yellow or brown. Fresh human bile has also a green colour, but that observed in the post-mortem or dissecting-room is always brown, because of this process of reduction. These two normally occurring bile pigments are related to each other in a manner analogous to hæmoglobin and oxyhæmoglobin; bilirubin ($C_{16}H_{18}N_2O_3$) on oxidation passes into biliverdin ($C_{16}H_{18}N_2O_4$).

Haycraft and Scofield[1] observed in the gall bladder itself reduction going on, as shown by the fact that, while the bile in the middle of the gall bladder was green, the thicker bile mixed with mucus near the bladder wall was orange-brown, and the mucous membrane itself of a brown colour. To this slow reduction Haycraft and Scofield ascribe also the presence of bilirubin and not biliverdin in the gallstones of oxen, although the latter is the chief pigment found in ox bile. Putrefaction readily brings about the same reduction in the bile pigments. Bile with the bilirubin tint predominant does not turn green from oxidation of this pigment to biliverdin, when it is exposed to the air, unless it be made strongly alkaline with caustic alkali. In this increased readiness to take up oxygen in alkaline solution, bilirubin resembles a large number of other organic substances, such as pyrogallol and pyrocatechin. Haycraft and Scofield were also able to induce these changes by the action of nascent oxygen and of ozone. Working with a battery of four or five Grove cells, and leading from platinum electrodes into brown-coloured bile (in a beaker or on filter paper), they found that the oxygen developed at the anode caused in a few minutes a change in colour of the bile, through green and blue into violet, followed by bleaching. On reversing the poles, so that reduction instead of oxidation took place at this spot, an inverse change in colour back to brown was observed.

Bilirubin and biliverdin have chemically the character of weak acids, as is shown by the ease with which they unite with bases to form salt-like bodies. Such compounds with alkalies are soluble in water, but with alkaline earths are insoluble; as, for example, the compound of bilirubin with calcium, which makes up the bulk of red gall stones, and forms a convenient source for the preparation of the pigment. Neither pigment has a spectrum showing absorption bands, but in each there is continuous absorption at the blue end of the spectrum.

Bilirubin has borne in the history of the bile pigments many names, such as cholepyrrhin, biliphäin, cholephäin, and bilifulvin; but, fortunately, all these names have now disappeared, and the properties of the substances described under them by different observers, so far as they have been substantiated, have been aggregated under one name and to one substance, bilirubin, the red colouring matter of bile. Bilirubin is a constant constituent of bile, and is found besides as a calcium compound in red gall stones. It is also present in traces in the serum of some animals. Hammarsten[2] found it in the serum of the horse. By precipitating the serum globulin with acetic acid, the pigment is thrown down with the globulin, and on drying the precipitate and extracting with chloroform, bilirubin is dissolved out. It seems, however, to be absent in

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiv. S. 173.
[2] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1878, Bd. viii. S. 129.

human serum and that of the ox. Most important, from the point of view of the origin of the bile pigments, is the discovery that the microscopic crystals often found in old blood clots and extravasations, and described by Virchow[1] as hæmatoidin, are usually bilirubin; this shows that the bile pigments are probably products of disintegration of hæmoglobin. Here it is needful to guard against mistaking lutein for bilirubin. The two substances may be distinguished by their solubilities. Both are soluble in chloroform, but bilirubin is thrown out of solution on the addition of an alkali (from the formation of a compound with the alkali insoluble in chloroform), while lutein is not so precipitated. Bilirubin is also found, in cases of jaundice, in the urine and in the tissues.

Bilirubin is best prepared from the gallstones of the ox, which are very common and easily procurable. The gallstones are washed, dried, powdered, and then extracted in turn with ether, boiling alcohol, and boiling water, to remove cholesterin (which is, however, rarely present in appreciable quantity in ox gallstones) and bile acids. The residue is treated with dilute hydrochloric acid, to set free the bilirubin from its calcium combination, washed with water and alcohol, and finally extracted with boiling chloroform, in which the bilirubin dissolves.

The chloroform is distilled from the extract, and the impure bilirubin is freed from an accompanying substance, bilifuscin, by digesting with absolute alcohol, in which this substance dissolves, and is then redissolved in chloroform. It is purified further by throwing out of concentrated solution in chloroform, by the addition of absolute alcohol, redissolving and reprecipitating repeatedly. It is lastly dissolved in as little as possible of boiling chloroform, from which it crystallises on cooling.

In amorphous condition, as when precipitated by alcohol and dried, bilirubin is an orange-coloured powder; when crystalline, it is of a dark red or reddish-brown colour, resembling chromic acid. The crystals are rhombic plates with rounded-off angles; they are more easily soluble in chloroform than in any other solvent; somewhat soluble in carbon bisulphide and amyl alcohol; nearly insoluble in ether, alcohol, turpentine, benzol, and glacial acetic acid. Bilirubin is soluble easily in alkalies and their carbonates, combining with them to form salts. Calcium chloride, added to these solutions, precipitates the calcium compound $(C_{16}H_{17}N_2O_3)_2Ca$ as a rust-coloured precipitate. Treated with sodium amalgam, bilirubin yields hydrobilirubin; on oxidation it passes into biliverdin and more highly oxygenated compounds. Several formulæ have been proposed for bilirubin;[2] the most generally accepted is that of Maly ($C_{16}H_{18}N_2O_3$). Bilirubin is oxidised in alkaline solution in the air to biliverdin in the same manner as bile, which owes this reaction to the bilirubin it contains.

Ehrlich's test.[3]—Ehrlich describes a colour test for bilirubin, which is not given by biliverdin. To a solution of bilirubin in chloroform an equal volume of a watery solution of diazobenzolsulphonic acid is added, and just enough alcohol to cause the two fluids to mix, when the fluid turns a beautiful red colour; on adding, drop by drop, concentrated hydrochloric acid, the colour of

[1] *Virchow's Archiv*, 1847, Bd. i. S. 379, 407. See also Robin, *Compt. rend. Acad. d. sc.*, Paris, 1855, tome xli. p. 506; Jaffé, *Virchow's Archiv*, 1862, Bd. xxiii. S. 192; E. Salkowski, *Hoppe-Seyler's Med.-chem. Untersuch.*, Berlin, 1868, S. 436.

[2] Städeler, *Ann. d. Chem.*, Leipzig, 1864, Bd. cxxxii. S. 323; Thudichum, *Journ. f. prakt. Chem.*, Leipzig, 1868, Bd. civ. S. 401; Maly, *ibid.*, Bd. civ. S. 28; Maly, *Ann. d. Chem.*, Leipzig, 1876, Bd. clxxxi. S. 106; Nencki u. Sieber, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1884, Bd. xvii. S. 2275.

[3] *Centralbl. f. klin. Med.*, Bonn, 1883, Bd. iv. S. 722; Krukenberg, "Chem. Untersuch.," 1886, S. 77.

the solution changes through violet into blue; if a layer of potassium hydrate solution is now introduced beneath the blue solution, there develops an (alkaline) bluish-green zone underneath, separated from the blue solution above by a red band where the reaction is neutral.

Biliverdin is present in all green-coloured biles, and may be obtained from them, by adding a solution of barium chloride, as a dark green precipitate, which may be washed with water and alcohol, and decomposed by dilute hydrochloric acid, when the biliverdin remains as a flocky precipitate; this is freed from fats by washing with ether, and is then dissolved in alcohol; on evaporating the alcohol, the biliverdin is left behind as a dark green scale. Biliverdin can best be prepared pure from an alkaline solution of bilirubin. This is allowed to oxidise by exposing to the air in a shallow dish, until it turns a brownish-green colour; the solution is then precipitated with hydrochloric acid, which sets free insoluble biliverdin from the soluble compound with the alkali; the precipitate is washed with water till free from hydrochloric acid, dissolved in alcohol, and reprecipitated by the addition of water. This precipitate is washed with chloroform to remove traces of bilirubin, and pure biliverdin remains behind, being insoluble in chloroform. It forms a very dark green amorphous powder, insoluble in water, ether, chloroform, carbon bisulphide, or benzol; but soluble with a fine green colour in alcohol, glacial acetic acid, or concentrated sulphuric acid. According to MacMunn,[1] there is a green pigment in ox bile which differs from that prepared as above, in being soluble in chloroform. Biliverdin does not easily crystallise; it is said to be occasionally obtainable, by evaporating a solution in glacial acetic acid, in rhombic plates with rounded angles. With alkalies, biliverdin forms soluble compounds, giving brownish-green solutions, from which biliverdin falls as a flocky precipitate on the addition of acids. Calcium, barium, and lead salts form insoluble compounds with biliverdin; these are thrown down as dark green precipitates on addition of solutions of the corresponding salts to an alkaline solution of biliverdin. By nascent hydrogen, biliverdin is converted through bilirubin into hydrobilirubin. Different formulæ are given by different authors for biliverdin. Städeler[2] calculated it as $C_{16}H_{20}N_2O_5$, from analyses by Heintz. Heintz himself[3] gives $C_{16}H_{18}N_2O_5$. This formula assumes that in passing from bilirubin to biliverdin, the molecule takes up water as well as oxygen ($C_{16}H_{18}N_2O_3 + H_2O + O = C_{16}H_{20}N_2O_5$), but the accuracy of this is denied by Maly,[4] both from analysis and the amount of increase in weight observed in passing from bilirubin into biliverdin. He gives the formula of biliverdin as $C_{16}H_{18}N_2O_4$, and this result is confirmed by Thudichum,[5] except that the latter halves the formula, giving $C_8H_9NO_2$.

Gmelin's test for bile pigments.—This very distinctive test for the bile pigments has already been mentioned. It depends upon the remarkable changes in colour accompanying the oxidation of bilirubin. In such oxidation the other normal bile pigment, biliverdin, is first produced; and this in turn, by further oxidation, is converted into a blue pigment, bilicyanin; after this follows, according to some, a purple pigment (bilipurpurin) before the final stage of oxidation to a yellow compound, choletelin. The production in series of these artificial products of oxidation is what constitutes Gmelin's test.

If either a solution of bilirubin or some diluted bile be carefully

[1] *Journ. Physiol.*, Cambridge and London, 1885, vol. vi. p. 25.

[2] *Ann. d. Chem.*, Leipzig, 1864, Bd. cxxxii. S. 323.

[3] *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1851, Bd. ii. S. 59; *Ann. d. Phys. u. Chem.*, Leipzig, 1851, Bd. lxxxiv. S. 106.

[4] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1874, Bd. iv. S. 302; and Hermann's "Handbuch," Bd. v. (2), S. 159.

[5] *Journ. f. prakt. Chem.*, Leipzig, 1868, Bd. civ. S. 220.

poured on the surface of fuming nitric acid in a test tube, so as not to mix the two liquids, a series of coloured zones appears in the lower part of the column of bile above the acid; next to the acid is the most oxidized product (choletelin), represented by a yellow-red zone; above this is a purer red, passing into a purple, which is replaced by a blue zone (bilicyanin); and, lastly, there is a very broad green zone, corresponding to the least oxidized product (biliverdin). The test may also be made by spreading out the bile in a thin film over the inside of a porcelain capsule and placing a drop of fuming nitric acid in the centre of this film, when a series of colours develop in the above order around the drop; or perhaps, most conveniently of all, according to Rosenbach's modification, by moistening a piece of filter paper in the suspected fluid, and placing a drop of fuming nitric acid in the centre.

Huppert's test.—Huppert's test consists in precipitating calcium bilirubinate, by the addition of milk of lime, or calcium chloride and ammonia, to a solution of an alkaline bilirubinate (or alkaline bile); after washing with water, the precipitate is boiled for some minutes with alcohol acidified with sulphuric acid, when, in presence of bile pigments, the solution develops an emerald-green or blue-green colour.

Bilicyanin is the name given to the substance present at that stage of oxidation of bilirubin by artificial means, such as fuming nitric acid, when the solution has a blue colour. The stage is a very transient one, and, though many have worked at the subject, no one has yet succeeded in isolating the substance to which the blue colour is due. It is probably an unstable oxidation product, intermediate between biliverdin and choletelin. A blue solution, which keeps for some hours, may be obtained by adding to a solution of bilirubin in chloroform a little nitric acid, and shaking till a violet tint first appears. Rectified spirit is then quickly added; this very much slows the completion of the oxidation, so that the blue colour is preserved for some time. If an ammoniacal solution of bilirubin be mixed with strong fuming nitric acid, a little at a time, and excess of acid removed each time by addition of ammonia, a dark flocky precipitate is obtained, from which biliverdin can be removed by alcohol, leaving behind a deep dark blue powder.[1] Heynsius and Campbell[2] have found that certain gall stones in man, after extraction with alcohol and ether, yield to dilute acids a violet-brown pigment, which they identified as bilicyanin spectroscopically.

Jaffé[3] first observed that the blue stage of the oxidation process gave an absorption spectrum; in strong solution, it shows a wide band beginning to the red side of D, and ending between D and E; on dilution this band resolves itself into two dimmer bands (α and β). As oxidation proceeds, a third band (γ) appears between *b* and F, whilst the two first mentioned gradually become fainter and disappear. This third band does not belong to the blue stage (bilicyanin), but to the substance formed in the final stage of the oxidation (choletelin). The violet colour obtained before the final permanent reddish brown is probably due to an admixture of the latter colour with blue.

Bilifuscin is a substance separated in the preparation of bilirubin from gall stones; very little is known of its properties or chemical relationships.

[1] Jaffé, *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, Bd. vi., S. 241; *Journ. f. prakt. Chem.*, Leipzig, 1868, Bd. civ. S. 401.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 537.

[3] *Loc. cit.*

In the presence of bilirubin it is soluble in chloroform, although difficultly soluble in this solvent alone; hence, after treatment with chloroform in preparing bilirubin, both substances come into solution. When the chloroform solution is concentrated, and excess of alcohol added, the bilirubin is precipitated, while the bilifuscin remains soluble, and is found in the alcoholic filtrate along with some cholesterin and higher fatty acids. After removal of the alcohol by evaporation, the residue is treated with ether, which dissolves out these impurities, and chloroform, which removes any traces of bilirubin left behind. The almost black, dark brown residue so obtained, was termed bilifuscin by Städeler;[1] who made incomplete analyses of it, from which he deduced the formula $C_{16}H_{20}N_2O_4$ (?). When quite pure, bilifuscin does not give Gmelin's reaction;[2] it is found in very old post-mortem bile[3] as well as in gallstones, but not in fresh bile. Bilifuscin has only been obtained in an amorphous form; it is soluble in alcohol and in alkalies; almost insoluble in water, ether, and chloroform; its relationship to bilirubin is unknown. The biliprasin of Städeler[4] is probably only a mixture of bilifuscin and biliverdin. *Bilihumin* is a name used by the same observer to designate a black mass taken up by strong solution of ammonia, from the residue of gallstones which have been thoroughly exhausted with chloroform, alcohol, and ether; it does not give Gmelin's reaction.

Hydrobilirubin ($C_{32}H_{40}N_4O_7$), a reduction product of bilirubin, is an important substance, from the connection it makes between the bile pigments, those of the urine and the products of disintegration of hæmoglobin.

Maly[5] first obtained it by the action of nascent hydrogen (from sodium amalgam) on an alkaline solution of bilirubin; biliverdin similarly treated also yields it, being first converted into bilirubin. At the end of the reaction the light brown coloured fluid is decanted from the mercury, and acidified with hydrochloric acid. On the addition of the acid the solution becomes much darker in colour, and abundant dark brown flocks of hydrobilirubin separate out; these are separated from the solution, dissolved in ammonia, reprecipitated with hydrochloric acid, and washed with water. After so washing away all the salts the pigment becomes less soluble in water. After drying it forms a dark reddish-brown powder, easily soluble in alcohol, or a mixture of alcohol and ether; not so soluble in ether alone. These solutions have, when concentrated a reddish brown, when dilute a rose colour. Chloroform dissolves it to form a yellowish-red solution. In alkalies it dissolves to a pale yellow solution, becoming red on the addition of an acid. Maly ascribes the yellow colour to a compound with the alkali, the red to the free substance.

Hydrobilirubin in solution has an absorption spectrum, showing a dark band between *b* and F. On addition of ammonia this band fades out, but reappears a little to the left on the addition of a trace of zinc chloride to the solution. This solution containing zinc chloride and ammonia has a rose colour and a green fluorescence. Hydrobilirubin once formed does not readily give Gmelin's test; that is to say, it is not easily oxidisable again to bilirubin or biliverdin.

Maly recognised his new substance as identical with a urinary

[1] *Vrtljschr. d. naturf. Gesellsch. in Zurich*, 1863, Bd. viii.
[2] Brücke, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1860, Bd. vi. S. 173.
[3] Simony, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, Bd. vi. S. 75.
[4] *Loc. cit.*
[5] *Jahresb. ü. d. Fortschr. d Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 232.

pigment, already described under the name of urobilin, and discovered by Jaffé [1] under pathological conditions in the urine. Immediately before Maly's discovery, Hoppe-Seyler [2] had described a brownish-red substance, which he obtained by the action of zinc and hydrochloric acid (*i.e.* nascent hydrogen), on hæmatin; this, he afterwards stated to be impure hydrobilirubin.[3] When one considers that bilirubin is poured into the intestine with the bile, and that it is here subjected to reducing influences, as is shown by the frequent presence of hydrogen in the intestinal gases, it is natural to suppose that a considerable conversion of bilirubin into hydrobilirubin goes on in the intestine. The pigments of the fæces, which must arise mainly from the bile pigments, do not give Gmelin's reaction; extraction of the fæces with dilute spirit, evaporating and extracting the residue with strong spirit, yields a solution which shows the spectrum of hydrobilirubin.[4] This pigment of the fæces had been already described as stercobilin by Vanlair and Masius.[5] Jaffé [6] considered it as identical with his urobilin. Maly gives the above theory of its formation in the intestine from bilirubin, and looks upon all three, as well as Hoppe-Seyler's compound from hæmatin, as one substance, namely, hydrobilirubin.[7]

It is generally accepted that these substances are closely related, if not identical, and their relationship is of the utmost importance in connecting the pigments of the bile with the waste products of hæmoglobin.[8]

Choletelin.—Besides this important reduction derivative of bilirubin, we also owe to Maly [9] the discovery of choletelin, the final substance obtained in its oxidation by nitric acid.

At the end of the reaction a yellow colour is obtained, not widely different from that of the bilirubin from which the reaction started; when this condition is reached, all the intermediate products have been converted by oxidation into one substance.

Choletelin is best prepared, according to Maly,[10] by leading a stream of nitrous fumes (prepared by acting on arsenious acid with nitric acid) through bilirubin suspended in alcohol. The fluid passes through the colours of Gmelin's reaction, and finally a clear, pale, yellowish-red solution is left; this is thrown into water, when choletelin separates out in rust-coloured flocks, which form, when dried, a brown powder. Choletelin is amorphous and probably represented by the formula $C_{16}H_{18}N_2O_6$; it is soluble in alcohol, ether, chloroform, and acetic acid. It is also soluble in alkalies, and precipitated from such solution by acids. In acid solution it shows a dim absorption band lying between *b* and *F*, and corresponding to the band γ observed by Jaffé in solutions of bilicyanin. In neutral alcoholic solution this band disappears.

[1] *Virchow's Archiv*, 1869, Bd. xlvii. S. 405–418.

[2] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1871, Bd. i. S. 80; *Med.-chem. Untersuch.*, Berlin, 1871, S. 536.

[3] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1874, Bd. vii. S. 1065.

[4] Maly, Hermann's "Handbuch," Bd. v. (2), S. 162.

[5] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1871, Bd. i. S. 229.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 537.

[7] Hermann's "Handbuch," Bd. v. (2), S. 162.

[8] See MacMunn, *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 71; Eichholz, *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 326; Garrod and Hopkins, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 113; Garvoch, *ibid.*, 1897, vol. xxi. p. 190.

[9] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1868, Bd. lvii. Abth. 2, S. 107; 1869, Bd. lix. Abth. 2, S. 602.

[10] Hermann's "Handbuch" Bd. v. (2), S. 165.

The halogens react energetically with bilirubin, forming substitution products. When bromine acts on bilirubin, a series of changes in colour take place, exactly counterfeiting those observed in Gmelin's reaction. The reactions are, however, quite different; and the process is not, as one might naturally have expected, an oxidation, but a substitution of bromine for hydrogen[1] ($2C_{16}H_{18}N_2O_3+3Br_2=C_{32}H_{33}Br_3N_4O_6+3HBr$).[2]

The reaction is best shown by adding a dilute solution of bromine in chloroform, cautiously, to a solution of bilirubin, also in chloroform. The solution changes through green, blue, and red into yellow, as the bromine is added, and may be stopped and examined at any stage. If chloroform free from alcohol be used, a tribromo derivative separates out of solution. Decanted from chloroform, dissolved in alcohol, and reprecipitated by adding water, this compound is obtained as a dark blue powder, soluble in alcohol, in ether, or in chloroform containing alcohol; but insoluble in pure chloroform, or in water. Alkalies split it up, yielding biliverdin.

Nothing is known of the chemical constitution of the bile pigments, and very little of the intermediate stages in their production from hæmoglobin. Their connection with hæmoglobin rests on—(1) The identity of hæmatoidin produced in old blood clots with bilirubin. (2) The identity of Hoppe-Seyler's reduction product obtained from hæmatin, and thus indirectly from hæmoglobin, with Maly's hydrobilirubin. (3) The absence of bile pigments in such animals as have no hæmoglobin.[3] (4) The fact that anything causing increased destruction of red blood corpuscles, or the intravenous injection of hæmoglobin, causes an increased secretion of bile pigments.[4] (5) Hæmatoporphyrin is isomeric with bilirubin, shows with nitric acid colour changes somewhat resembling Gmelin's reaction, and yields on reduction with nascent hydrogen a substance closely resembling and probably isomeric with hydrobilirubin. According to Nencki and Sieber,[5] bilirubin is formed in the liver by the hæmoglobin first splitting up into hæmatin and proteid. The hæmatin thus formed then takes up water, loses its iron, which is retained in combination in the liver, and so forms bilirubin, thus—

$$\underset{\text{(hæmatin)}}{C_{32}H_{32}N_4O_4Fe} + 2H_2O - Fe = \underset{\text{(bilirubin)}}{C_{32}H_{36}N_4O_6}\text{; or } 2(C_{16}H_{18}N_2O_3)$$

Direct experiments on the formation of bile pigments from hæmoglobin apart from the liver have been carried out by Latschenberger.[6] If the corpuscles and serum of horse blood be separately injected subcutaneously at different parts in the horse, and after the lapse of about twelve days the animal be killed, and the parts where the injections have been made are examined, it is found that while, at the part where the

[1] Thudichum, *Journ. Chem. Soc.*, London, 1875, vol. xxviii. p. 389; Maly, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. lxxii. Abth. 2.

[2] Maly, Hermann's "Handbuch," Bd. v. (2), S. 167.

[3] Hoppe-Seyler, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 399. See, however, Krukenberg, *Centralbl. f. d. med. Wissensch.*, Berlin, 1883, No. 44, S. 785.

[4] Frerichs, *Arch. f. Anat. u. Physiol.*, Leipzig, 1856, S. 59; W. Kühne, *Virchow's Archiv*, 1858, Bd. xiv. S. 310; Nothnagel, *Berl. klin. Wchnschr.*, 1866, vol. vi. S. 31; Tarchanoff, *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. ix. S. 329; Minkowski and Bassorin, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxiii. S. 145.

[5] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1884, Bd. xvii. S. 2275; *Monatsh. f. Chem.*, Wien, 1888, Bd. ix. S. 115; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1888, Bd. xxiv. S. 430.

[6] *Monatsh. f. Chem.*, Wien, 1888, Bd. ix. S. 52; *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1888, Bd. xcvii. Abth. 2b, S. 15.

serum has been injected the tissues present a normal appearance and contain no bile pigments, on the other hand, round the spot where the corpuscles have been injected, the tissues contain, besides fluid blood, a substance in flakes, varying in colour from dark orange to bright yellow, composed of small spherical masses about a quarter of the size of red corpuscles, which give Gmelin's reaction very readily. The same result may be obtained on injecting crystallised hæmoglobin, suspended in water; here granular masses of *a greenish-yellow* colour are obtained, which also give Gmelin's reaction.

Spectra of bile.—A considerable amount of continuous absorption at both ends of the spectrum is found on examining the bile of any animal, but in some animals the bile also shows well-marked absorption bands.[1]

Cholohæmatin.—The most characteristic of these band-spectra is that exhibited by ox or sheep bile which has stood for some time in contact with air. This spectrum, according to MacMunn,[2] "presents in a deep layer three bands, in a thinner one four bands, and in a still thinner a fifth band at F is visible." The spectrum is well seen in an alcoholic solution of evaporated ox bile. Of the four well-marked bands, two lie close to the D line, on either side of it; a third lies in the red, immediately to the right of the C line; and the fourth covers the E and b lines. No pure material has yet been isolated, so that it is not even known whether the spectrum is due to one or several substances. MacMunn[3] has obtained an amorphous residue of a dark sap-green colour, containing abundantly material which gives the spectrum, by treating ox bile with absolute alcohol and acetic acid, alternately dissolving in chloroform and ether, and washing the chloroform solution with water. This material has been named cholohæmatin by MacMunn, from its occurrence in bile and its supposed origin from hæmatin.

The spectrum is not exhibited by *fresh* ox or sheep bile,[4] but is first developed on standing in contact with air, probably from a chromogen present in the fresh bile. The bands near D first appear, to be followed much later by the other two; the appearance of the spectrum is not a result of putrefaction.[5]

MacMunn[6] obtained a spectrum closely resembling that of hæmatoporphyrin by the action of sodium amalgam on cholohæmatin, prepared as above indicated, from which he argues that the latter is a derivative of hæmatin.

The fresh bile of the mouse shows a well-marked band at F, corresponding to the urobilin band; and more or less distinct bands in the same position in the bile of other animals indicate, according to MacMunn, traces of urobilin in these fluids. Characteristic absorption-band spectra are also found in the bile of the guinea-pig, pig, rabbit, and crow. Human bile shows no bands, but an alcoholic extract exhibits a well-marked band at D; these, as well as the spectra of Gmelin's and Pettenkofer's reactions, are shown in Plate III. at the end of this volume.

Other constituents of bile.—Besides the bile salts and bile pigments, which are normally found only in the bile, other constituents are present which are also found in other parts of the body; these are cholesterin, fats, soaps, and lecithin, besides minute traces of urea and of the diastatic ferment already mentioned.

[1] Bogomoloff, *Centralbl. f. d. med. Wissensch.*, Berlin, 1869, vol. vii. S. 530.
[2] "The Spectroscope in Medicine," London, 1880, p. 158.
[3] *Journ. Physiol.*, Cambridge and London, 1885, vol. vi. p. 24.
[4] Bogomoloff, *loc. cit.*; Heynsius and Campbell, *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 540.
[5] Gamgee, "Physiological Chemistry," London, 1893, vol. ii. p. 333; see also Hoppe-Seyler, *loc. cit.*
[6] *Loc. cit.*, p. 27.

Cholesterin.—The amount of cholesterin in bile is very variable, ranging from 0·5 to 5 per cent. Cholesterin is insoluble in water or dilute saline solution, and is dissolved in bile by the agency of the bile salts, in solutions of which it is easily soluble. When the amount of bile salts is insufficient to hold it in solution, it slowly passes out of solution in a concretionary form around any particle of foreign matter present in the bile, or around an existing concretion forming in this manner a variety of gall stone.

According to Hoppe-Seyler, cholesterin is a cleavage product, constantly formed in the metabolic changes of the living cell; and for this reason it is that cholesterin is invariably found as a chemical constituent of both animal and vegetable cells. Cholesterin does not easily undergo decomposition in the animal organism when once formed, and is principally excreted in the higher animals in the bile. It is found in increased quantity in tissue which is undergoing pathological change; this may, perhaps, be due to increased inability on the part of the cells in their vitiated condition to break up the stable cholesterin. Cholesterin is found in largest quantity as a constituent of the myelin of nerve fibres and in the blood corpuscles. It is probably formed most in the metabolism of nerve tissue, taken up by the liver cells from the blood, and passed as an excretion into the bile ducts.

Cholesterin is purely an excretion, and is not reabsorbed, but passes out of the body with the fæces. This is also the fate of the bile pigments, which are gradually reduced to hydrobilirubin (stercobilin) in their passage along the intestine. This substance may easily be extracted from the fæces by absolute alcohol, after making acid with sulphuric acid. The bile pigments have a poisonous action when injected into a vein, which indicates that if they are reabsorbed at all they must be changed in the process.[1]

Lecithin.—The amount of lecithin present in bile is much greater than in any of the other secretions. All the lecithin, and any direct products of its decomposition to be removed from the body, are carried off in the bile. As lecithin, as well as cholesterin, is one of the constituents of nerve tissue, the liver, by means of the bile, may be looked upon as the great channel for the removal of the products of nervous metabolism. Lecithin is also held in solution by the bile salts.

Reabsorption of bile salts—Their functions in the organism.—The bile salts differ from the other biliary constituents in that they are not purely an excretion. They are to a large extent reabsorbed, and undergo a circulation in the body, with the probable function of acting as carriers for the otherwise insoluble cholesterin in the bile. Such an absorption of bile salts has been shown to take place in different ways, which are, briefly, as follows:—

1. Bile salts taken by the mouth, cause an increased flow of bile; indeed, from recent observations by various experimenters,[2] it seems that the bile salts are the only substances which truly act as cholalogues. This action can only be due to their absorption followed by an increased elimination of them by the liver.

2. The bile of the dog contains only taurocholates. If unpaired

[1] De Bruin, *Centralbl. f. klin. Med.*, Bonn, 1890, Bd. xi. S. 491.

[2] Baldi, *Arch. ital. de biol.*, Turin, 1883, tome iii. p. 395; Paschkis, *Schmidt's Jahrb.*, Leipzig, 1885, Bd. ccvi. S. 19; Nissen, *Centralbl. f. d. med. Wissensch.*, Berlin, 1890, Bd. xxviii. S. 948.

cholalic acid be given by the mouth, the amount of bile is increased, but still only taurocholates are found in the bile; but Weiss[1] found, after giving sodium glycocholate for three days (5–9 grms. per diem), that the bile in the gall bladder at death contained glycocholates, amounting to 25–30 per cent. of the total bile salts present.

3. No connection exists between the amount of proteid metabolism and the amount of cholates produced, such as would be found if the cholates were a channel for the excretion of the nitrogen and sulphur of proteid decomposition products.[2]

4. Tappeiner[3] identified bile salts in chyle obtained from the thoracic duct in the dog.

5. Bidder and Schmidt[4] only found cholalic acid in traces in the fæces.

A review of all these facts shows that the bile salts are not an excretion, but perform a circulation in the body. Besides the function of dissolving the cholesterin to be excreted, the bile salts are also credited with the effect produced by bile in aiding the absorption of fats.[5] Again, bile salts dissolve insoluble soaps of the alkaline earths. This may be shown by precipitating a soluble soap with calcium or magnesium sulphate, and then adding a solution of bile salts and gently warming when the precipitate dissolves.[6] Maly and Emich state that taurocholic acid completely precipitates native proteids, but not albumoses or peptones.[7] This in part explains the precipitation observed when a solution in which peptic digestion is going on (or gastric chyme) is mixed with bile; but part of the precipitate is doubtless mucin from the bile itself. The subject has been investigated by Hammarsten,[8] who found that syntonin was completely, peptone only partially, precipitated from an acid solution in which peptic digestion of hard-boiled white of egg had been carried out, by the addition of bile from which the mucin had been removed by alcohol. Hammarsten supposes that the purpose of this precipitation of the semi-digested proteid, which must occur in natural digestion when the gastric chyme comes in contact with the bile, is that it may, by adhering to the intestinal wall, be longer subjected to intestinal digestion than it would be if it remained in solution.

[1] *Centralbl. f. d. med. Wissensch.*, Berlin, 1885, Bd. xxiii. S. 121. Similar results have been obtained by Prévost and Binet, *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvi. p. 1690; Winteler, Inaug. Diss., Dorpat, 1892.

[2] Kunkel, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 344; Spiro, *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, Supp. Bd. S. 50.

[3] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1878, Bd. lxxvii. Abth. 3.

[4] "Die Verdauungssäfte," S. 217.

[5] *Vide* "Fat Absorption," p. 454.

[6] Neumeister, "Lehrbuch d. physiol. Chem.," Jena, 1893.

[7] *Monatsh. f. Chem.*, Wien, 1883, Bd. iv. S. 89; 1885, Bd. vi. S. 95.

[8] *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1870, Bd. 1, S. 106. See also Chittenden and Cummins, *Am. Chem. Journ.*, Baltimore, 1885, vol. vii. p. 36; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1885, Bd. xv. S. 319.

DIGESTION OF CARBOHYDRATES.

The digestion of carbohydrates is brought about by the action of two distinct classes of enzymes, namely—1. Those which act on starches, producing sugars and dextrins: these are called amylolytic or diastatic ferments. 2. Those which act on various saccharoses, producing glucoses: these are called inverting ferments.

The two chief amylolytic ferments found in the digestive juices are ptyalin and amylopsin. The action of these ferments on starch may be demonstrated by adding to starch paste, either saliva or pancreatic juice, or a watery infusion of salivary or pancreatic gland. The paste very soon becomes quite fluid, and if the fluid be tested chemically for starch, it will be found that this substance is rapidly disappearing, and that a reducing material is being formed in continuously increasing amount in the solution. This testing may be done by removing a drop of the solution at intervals, and mixing it with a drop of a solution of iodine. At first the deep blue colour given by starch is obtained; this is replaced after a time by a violet, this again by a red colour, and finally no coloration at all is obtained. If at each of these stages portions of the solution be tested with Fehling's solution, it will be found that it has acquired reducing power, and that the amount of reduction increases with the length of time during which the action goes on.

The diastase of malt is very similar in its action to both these ferments, but is not identical with either of them, as is shown by the fact that while ptyalin and amylopsin act best at body temperature, the optimum temperature for the action of malt diastase is about 55° C.

Products of digestion of starch. — Whether the ferments are identical or not, their action, according to all observers, is the same. It was shown by Leube,[1] in 1831, that saliva dissolves starch-paste and forms sugar, and the same was shown for pancreatic juice by Bouchardat and Sandras,[2] in 1845. It was for many years believed that the action of these ferments was closely analogous to that of mineral acids, and that the sugar produced was grape-sugar. Dextrin was supposed to be the first stage in the process of saccharification, and from the dextrin it was thought that grape-sugar was afterwards formed. Musculus[3] was the first to show that all the starch was not so converted into sugar, but that saccharification only proceeded until the solution gave no longer a colour reaction with iodine; on adding fresh starch-paste, the reaction recommenced and proceeded as before, until again all colour reaction with iodine had vanished, when, as before, the reaction slackened and stopped, although there remained plenty of dextrin in the solution.

According to the earlier work of Musculus, the quantitative relationship in which the sugar and dextrin stand at the end of the reaction is, one part of sugar to two of dextrin; his later papers gave the reaction as stationary, when approximately equal quantities of sugar and dextrin are present in the solution.[4]

[1] *Arch. f. d. ges. Naturl.*, Nürnberg, 1831.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1845, tome xx. p. 1085.
[3] *Journ. de pharm. et chim.*, Paris, 1860, Sér. 3, tome xxxvii. p. 419.
[4] Payen, *Chem. Centr.-Bl.*, Leipzig, 1865, S. 845; Schwarzer, *ibid.*, 1870, S. 295; Schulze u. Marker, *ibid.*, 1872, S. 823.

Still later, Sheridan Lea,[1] working with much more dilute solutions than were usually employed by other experimenters (0·4 to 4 per cent.), found as much as 85 per cent. of the starch converted into sugar; and by more closely approximating the conditions of experiment to those of natural digestion, by carrying out the experiment in a dialyser instead of in a glass vessel, obtained a still greater reduction in the percentage of dextrin formed (7 to 8 per cent.). He is therefore of the opinion that in the alimentary canal starch is completely converted into sugar before absorption. The increase in sugar formation, due to removal of the products of digestion, was more marked in working with strong than in working with dilute solutions; this also goes to show that it is the accumulation of maltose in the solution which slackens and stops the reaction. All chemical reactions involving hydration, such as saponification of esters, become stationary at a determinate point when a fixed proportion of hydration has taken place; and this point is rigorously determined by the concentration in the solution of the various factors in the reaction. If the substance or substances formed in the reaction be continuously removed from the solution, or changed in nature as they are formed, the reaction proceeds to completion; but if the products of the reaction remain in solution unchanged, at a perfectly fixed point, dependent on the concentration in solution of each of the reacting substances, equilibrium is established, and no further change in the composition of the solution takes place.

On the other hand, Musculus and Gruber[2] claim to have isolated a dextrin after acting on starch paste with diastase for five days, by precipitating the dextrin with alcohol; on this dextrin, diastase, *even in the absence of the sugar*, has no further action.

In 1872, O'Sullivan[3] rediscovered the sugar described by Dubrunfaut[4] as formed by the action of malt extract on starch paste, isolated it, investigated its properties, and named it *maltose*. When it was so shown that the sugar formed by the action of malt diastase is not grape-sugar, attention was directed naturally to the sugars similarly formed by the action of the digestive enzymes of the saliva and pancreatic juice. Nasse[5] stated that the sugar formed by the action of saliva is not dextrose, but another sugar of different reducing power, to which he gave the name of *ptyalose*, which, however, was not maltose, as its reducing power was doubled on boiling with acids, while that of maltose was only increased by one-half. Soon after, v. Mering and Musculus[6] conclusively showed that the sugar really formed both by ptyalin and amylopsin is identical with O'Sullivan's maltose, and these results have been abundantly confirmed by subsequent observers. This result they established by the amount of increase of reducing power and reduction of rotating power, following boiling with a dilute mineral acid, as well as by the observation of birotation in the solution after boiling, which could only be due to the formation of grape-sugar. Such an analysis is rendered easy by the widely different specific rotatory powers and reducing powers of the two sugars (see table, p. 396).

The action of malt diastase, ptyalin, or amylopsin on starch paste takes place in several stages, corresponding to which more or less

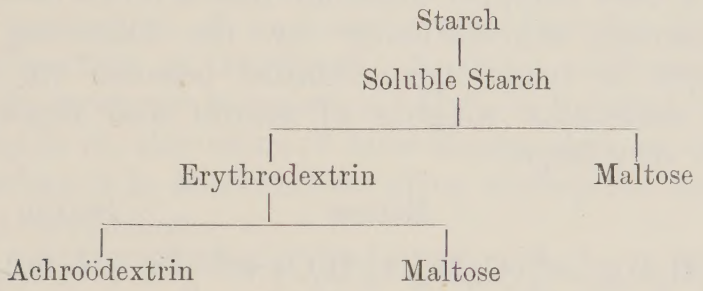
[1] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 226.
[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 187.
[3] *Journ. Chem. Soc.*, London, 1872, vol. xxv. p. 579.
[4] *Ann. de chim. et phys.*, Paris, 1847, tome xxi. p. 178.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 477; see also Seegen, *Centralbl. f. d. med. Wissensch.*, Berlin, 1876, S. 851.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 395; 1878, Bd. ii. S. 403. See also Brown and Heron, *Proc. Roy. Soc. London*, 1880, vol. xxx. p. 393.

well-differentiated substances have been described. The first step in the action is, according to all observers,[1] the formation of *soluble starch* (amigdulin, amidulin, amidogen, or amylodextrin). This substance is rapidly formed, usually in one or two minutes; it gives the same blue reaction with iodine as raw starch or starch paste, and is precipitated by tannic acid, by which it is distinguished from the dextrins formed in the subsequent stages.

In the second stage, this soluble starch is decomposed into a substance giving a red colour with iodine and maltose. The substance giving the red colour now gets the name given by Brücke of erythrodextrin; it corresponds to Nasse's dextrin, Griessmayer's dextrin-1 and Bondonneau's dextrin-α.

In the third stage, this erythrodextrin is split up into a dextrin (or several dextrins), giving no coloration with iodine, and hence called achroödextrin, and a further quantity of maltose. Finally, according to some, a part of this achroödextrin breaks up, yielding more maltose, and a variety of achroödextrin, altogether unaffected by diastatic ferments, which with the maltose split off at different stages from the intermediate products, forms the final product of the reaction, no matter how long prolonged.

These successive changes may be represented as in the following scheme:—



All observers are agreed as to the existence of soluble starch, and practically all as to that of erythrodextrin, although Musculus and Meyer[2] state that, on carefully mixing dextrin stained with iodine, with soluble starch stained with iodine, they obtained the colour of erythrodextrin, and conclude that what has been called erythrodextrin is probably such a mixed colour. This result has not been confirmed by other observers; still it should be borne in mind that a pure substance has not yet been isolated, and that at present erythrodextrin is only a name given to a substance *supposed* to exist, because of a red colour which is obtained at a certain stage in the digestion of starch by diastatic enzymes. The material which is found later in the process, which is not a sugar and gives no coloration with iodine, has been called achroödextrin, but it has none of the constant properties of a pure simple substance, and is probably a mixture of several substances (achroödextrins), though as yet none of these have been properly isolated.

Musculus and Gruber,[3] working on starch solutions with dilute acids and with diastase, differentiated, according to varying conditions of temperature, amount of diastase added, and length of time of action of the ferment, three achroödextrins (α, β, and γ), possessing, according to these observers, different

[1] Nasse, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 474; Griessmayer, *Chem. Centr.-Bl.*, Leipzig, 1871, S. 636; Brücke, "Vorlesungen," and *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1872, Abth. 3; Bondonneau, *Compt. rend. Acad. d. Sc.*, Paris, 1875, tome lxxxi. pp. 972, 1210; Musculus, *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 177.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 451.

[3] *Ibid.*, 1878, Bd. ii. S. 177.

specific rotatory powers and reduction coefficients; but they scarcely give adequate proofs that they are describing pure substances.[1] They found that even after twelve months a portion of the dextrin remained unconverted into maltose, and the substance so remaining was unfermentable by yeast. This substance is γ-achroödextrin, and is formed together with some maltose by the splitting up of β-achroödextrin, which in its turn is formed by a similar decomposition of α-achroödextrin.

The following is a summary of their results:—

	Sp. Rotatory Power.	Relative reducing Power for Fehling's Solution.	Reaction with Iodine.
Soluble starch	218°	6	Reddish blue.
Erythrodextrin . . .	...	...	Red.
α-Achroodextrin . . .	210°	12	No coloration.
β ,, ...	190°	12	,,
γ ,, ...	150°	28	,,
Maltose	150°	66	,,
Grape-sugar	50°	100	,,

The digestion of starch by diastatic enzymes consists of a breaking up, through several more or less well-defined stages, by a process of gradual hydrolysis, of a very complex molecule into a much simpler one, and might be represented schematically by the following general equation, which cannot be made more definite, because we are unacquainted with the molecular weights of starch and dextrin, only knowing that they are very large—

Starch Maltose Dextrin

$$(C_6H_{10}O_5)_n + (H_2O)_m = \frac{m}{2}(C_{12}H_{22}O_{11},H_2O) + \frac{n-m}{p}(C_6H_{10}O_5)_p$$

That is, starch and water, in presence of a suitable ferment, yield maltose and different dextrins, but we are ignorant of the value of n, m, and p.

Attempts to carry too far the analogy between the action of dilute mineral acids and that of the diastatic ferments on starch, led, as already stated, to an error, which persisted for several years, as to the products of the latter action. Nevertheless, a close analogy does exist between the two processes; both are essentially hydration processes; and in both the same stages may be observed. They only differ in two respects, first, that the dilute acid at boiling temperature acts much more rapidly; secondly, that it proceeds a stage further, and very rapidly converts the maltose formed into grape-sugar.

These successive changes may be best observed by boiling with very dilute acid (·2 per cent. or less). Soluble starch is first formed, giving, on neutralisation, a blue with iodine; next, is an intermediate stage, in which a violet is obtained followed by a stage giving a red colour (erythrodextrin); and finally a stage is reached at which a coloration is no longer obtained

[1] According to Brown and Morris (see *Trans. Chem. Soc.*, London, 1885, p. 527; 1889, p. 462), the chemical and physical properties of these different achroödextrins might be given by a variable mixture of one achroödextrin possessing no reducing power with maltose. They admit the existence besides achroödextrin, of *maltodextrin* (Herzfeld), a body intermediate between achroödextrin and maltose, but more nearly allied to the latter.

(achroödextrin). If, when this stage is reached, the solution is rapidly cooled and neutralised, a little maltose can be separated from accompanying dextrose, showing that maltose is here also formed, but is converted rapidly into grape-sugar.

Although maltose is the chief sugar formed in the action of both ptyalin and amylopsin upon starch, yet a trace of grape-sugar is also formed.[1] The quantity of grape-sugar formed is in both cases small, but is greater in the case of the pancreatic ferment. It has recently been discovered that in both salivary and pancreatic digestion, besides maltose and small quantities of grape-sugar, another sugar, isomaltose,[2] is formed, in considerable quantity. The relative quantity of the three sugars varies with the quantity of ferment present, and the duration of the experiment. A weak ferment and short time of action favour the formation of isomaltose; by much ferment and prolonged action large quantities of maltose are produced, accompanied by traces of dextrose.[3] It is stated that traces of an inverting ferment are present, both in the salivary and pancreatic glands, especially the latter; and it is possible that the traces of dextrose formed may be due to the action of these on the maltose and isomaltose first formed.

The action of the amylolytic enzymes on glycogen is precisely similar to their action on starch; dextrin, maltose, and isomaltose being formed in very much the same proportions.[4]

The production of maltose by the diastatic ferments is not the end of the digestion of amyloses; there is evidence that maltose never reaches the systemic circulation. If it be injected intravenously it is soon discoverable in the urine;[5] this shows that in digestion it is inverted, either before it is absorbed, or after absorption and before reaching the systemic circulation.

This further process of hydrolysis may be to some extent carried out, so far at least as concerns that portion of maltose arising from salivary digestion, by the hydrochloric acid of the gastric secretion, but it is mainly brought about by an inverting ferment, discovered by Brown and Heron in the mucous membrane of the small intestine, and also in the succus entericus.[6]

The succus entericus (as well as the intestinal mucous membrane and glycerin or water extracts of it) possesses only a feeble diastatic action

[1] Musculus and Gruber, *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 177; Musculus and von Mering, *ibid.*, 1878, Bd. ii. S. 403; von Mering, *ibid.*, 1881, Bd. v. S. 185; Brown and Heron, *Ann. d. Chem.*, Leipzig, 1879, Bd. cxcix. S. 165; 1880, Bd. cciv. S. 228; *Proc. Roy. Soc. London*, 1880, p. 393.

[2] Külz u. Vogel, *Ztschr. f. Biol.*, München, 1894, Bd. xxxi. S. 108. The existence of isomaltose is, however, denied by Brown and Morris (*Trans. Chem. Soc.*, London, 1895, vol. lxvii. p. 737), who state that it is a mixture of maltose and dextrins.

[3] *Journ. Physiol.*, Cambridge and London, vol. xv.; Abelous, *Compt. rend. Soc. de biol.*, Paris, 1891.

[4] Hensen, *Verhandl. d. phys.-med. Gesellsch. zu Würzburg*, 1856, Bd. vii. S. 219; *Virchow's Archiv*, 1857, Bd. xi. S. 395; Claude Bernard, *Gaz. méd. de Paris*, 1857, No. 13; J. Seegen, *Centralbl. f. d. med. Wissensch.*, Berlin, 1876, S. 849; *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix. S. 106; Külz u. Vogel, *Ztschr. f. Biol.*, München, 1894, Bd. xxxi. S. 108.

[5] Bimmermann, *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xx. S. 201; Philips, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1881, Bd. xi. S. 60; Dastre et Bourquelot, *Compt. rend. Acad. d. sc.*, Paris, 1884, tome xcviii. p. 1604; Bourquelot, *Journ. de l'anat. et physiol.*, etc., Paris, 1886, tome xxii. p. 161.

[6] Brown and Heron, *Proc. Roy. Soc. London*, 1880, p. 393; *Ann. d. Chem.*, Leipzig, 1880, Bd. cciv. S. 228; Vella, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1881, Bd. xiii. S. 40; Bourquelot, *Compt. rend. Acad. d. sc.*, Paris, 1883, tome xcvii. p. 1000.

on starch,[1] but has a remarkable power in converting maltose into grape-sugar. Brown and Heron surmised from this that maltose would be found to be a non-assimilable substance; unknown to them, Bimmermann had already shown this to be the case, and many subsequent observers have confirmed the result.

Digestion of cane-sugar.—Cane-sugar resembles maltose in not being directly assimilable from the blood; after intravenous injection it is excreted by the kidneys. In the course of digestion it is either completely inverted while in the alimentary canal, or may in part be so changed in its passage through the absorbing cells of the mucous membrane.

Some cane-sugar is inverted in the stomach, probably by the action of the hydrochloric acid there present.[2] Lehmann[3] repeatedly found, in the stomach and duodenum of rabbits fed on beetroot, invert-sugar only. Seegen found that, after feeding dogs on cane-sugar, the stomach always contained a small amount of a reducing sugar along with a great deal of unchanged cane-sugar; and that the small intestine contained no sugar. He argues from this, that all the cane-sugar is inverted in the stomach, the invert-sugar being there absorbed as fast as it is produced. It is probable, however, from the work of other observers, that a considerable part of the inversion takes place in the small intestine by the action of the intestinal juice, and it may be also by the direct action of the cells of the intestinal mucous membrane.

Watery infusions of the mucous membrane from any part of the small intestine are capable of inverting cane-sugar,[4] and the same power is possessed by the intestinal contents in animals which have been killed during active digestion.[5] The intestinal juice obtained by Vella[6] from fistulæ almost instantly inverted cane-sugar, and a strong inverting action of pure succus entericus has been observed by many others.

The inversion of the saccharoses by the intestinal juice is brought about by enzymic action, but very little is known of the enzyme or enzymes involved. It was supposed by Hoppe-Seyler and Thierfelder[7] that the inversion might be due to bacterial action or to inverting enzymes taken in with the carbohydrate food, but the former

[1] A diastatic action on starch was found by Schiff, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1867, Bd. i. S. 155; Eichhorst, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1871, Bd. i. S. 198; Paschutin, *ibid.*, S. 304; Ewald, *Virchow's Archiv*, 1879, Bd. lxxv. S. 409; Garland and Masloff, *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1878, Bd. ii. S. 290; Brown and Heron, *loc. cit.*; Dana, *Med. News*, Phila., 1882, vol. xli. p. 59.; Hamburger, *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lx. S. 560; Mendel, *ibid.*, 1896, Bd. lxiii. S. 425. On the other hand, its existence is denied by Thiry and by Leube, *Jahresb. ü. d. Fortschr. d. ges. Med.*, Erlangen, 1868, Bd. i. S. 97. It must be remembered that the diastatic action is admittedly a slight one by most of those observers who confirm it, and that most organs and tissues possess a slight diastatic action, so that it is difficult to be certain that the intestinal mucous membrane specifically secretes a diastatic ferment.

[2] It is certain, from purely chemical experiments, that the acid is capable of producing such an effect, and no inverting enzyme has ever been shown to exist in the gastric secretion.

[3] "Lehrbuch der physiol. Chem.," Aufl. 2. Bd. iii. S. 255; v. Becker, *Ztschr. f. wissensch. Zoologie*, Bd. v. S. 123; J. Seegen, *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. xl. S. 41.

[4] Paschutin, *Arch. f. Anat. u. Physiol.*, Leipzig, 1871, S. 306.

[5] Claude Bernard, "Leçons sur la diabète et la glycogenese animale," Paris, 1877, 257–261.

[6] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1889, Bd. xiii. S. 62; see also Bastianelli, *ibid.*, 1886, Bd. xiv. S. 146.

[7] "Handbuch der. path. u. physiol. chem. Analyse," 1893, Aufl. 6, S. 298. See also Pautz and Vogel, *Ztschr. f. Biol.*, München, 1895, Bd. xxxii. S. 304.

supposition is negatived by the rapidity of the action, and its progress in presence of antiseptics; and both by the recent observations of Miura,[1] which show that the mucous membrane of the intestine of newly-born animals, under antiseptic conditions, causes inversion. No inversion was obtained with the mucous membrane of the stomach or large intestine.

Brown and Heron[2] have shown that the dried mucous membrane of the small intestine is much more powerful, both in its diastatic action on starch and in its inverting action on cane-sugar and maltose, than are infusions of the same material. Starch also disappears from an intestinal fistula (Thiry) much more rapidly than it is possible for the succus entericus,[3] judging from other experiments, to convert it into sugar. These facts point to a possibility that the epithelial cells of the intestinal mucous membrane may possess the power of absorbing starches and saccharoses, and submitting them to diastatic and inverting processes, in passing them on to the lymph spaces of the villi; that, in fact, cellular digestion of absorbed carbohydrates may take place in the epithelial cells after absorption.

The secretion of the small intestine is generally stated to be inactive towards lactose, so that the inversion of this sugar probably occurs after its absorption by the columnar cells.[4]

Human succus entericus has been investigated by Ewald,[5] by Demant,[6] and by Tubby and Manning;[7] they all agree as to its diastatic action on starch and inverting action on cane-sugar. Tubby and Manning also tested its action on maltose, and found that this was converted into dextrose. The ferment or inverting material adhered to mucus whenever a precipitation of this took place in the fluid, so that the mucus was more effective than the clear fluid.[8]

DIGESTION OF PROTEIDS.

The digestion of proteids is a much more complex process than that of either the fats or carbohydrates, and one of which our knowledge is still less exact. In the digestion of carbohydrates we are absolutely certain that we have to do with a hydrolytic process, and that from a body of absolutely fixed percentage composition, though often of unknown molecular weight, there is produced in digestion a substance of known formula, and to a certain extent of known structure. In proteid digestion, while it is probable that a very similar action is taking place, we have no such certainty. The digestive process begins with material, the different proteids, which varies considerably in percentage composition.

[1] *Ztschr. f. Biol.*, München, 1895, Bd. xxxii. S. 266.

[2] *Proc. Roy. Soc. London*, 1880, vol. xxx. p. 399. See also Shore and Tebb, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. (*Proc. Physiol. Soc.*), and M. C. Tebb, *ibid.* vol. xv. p. 421.

[3] Röhmann, *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. xli. S. 424.

[4] Meyer, "Die Lehre von den chemischen Fermenten," 1882; Dastre, *Arch. de physiol. norm. et path.*, Paris, 1890, tome xxii. p. 103; C. Voit and Lusk, *Ztschr. f. Biol.*, München, 1891, Bd. xxviii. S. 275; Mendel, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 425. See, however, Pautz and Vogel, *Ztschr. f. Biol.*, München, 1895, Bd. xxxii. S. 304; Röhmann u. Lappe, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1895, Bd. xxviii. S. 2506.

[5] *Virchow's Archiv*, 1879, Bd. lxxv. S. 409.

[6] *Ibid.*, S. 490.

[7] *Guy's Hosp. Rep.*, London, 1891, vol. xlviii. p. 271; *Centralbl. f. d. med. Wissensch.*, Berlin, 1892, S. 945.

[8] Paschutin (*loc. cit.*) found that the inverting enzyme was mechanically precipitated along with collodion.

From this variable material products showing minute variations are produced, of which we only know that they are more soluble than the mother substance, less easily thrown out of solution by various precipitants, and to a certain slight extent are capable of diffusing through membranes.

Here our knowledge at present stops. In spite of most laborious researches on the subject by a host of observers, we know no more of the structure of any save the final decomposition products of proteid digestion than we do of the proteids themselves. Certain products have been isolated at various intervals in the progress of digestion of proteids, which show that the process gives rise to several intermediate bodies, ever increasing in solubility towards precipitants as they are formed nearer the end of the process; and it may be—it is a probable inference from analogy—that these substances are simpler than the proteids from which they originate, but as yet the simplest of them is too complex for our fragmentary knowledge to give any indication of its structure. Nor is there any knowledge of the relationship of these several stages of proteid digestion to one another.

It is very probable that the process of proteid digestion, like all the other digestive processes, is one of continuous absorption of the elements of water or hydrolysis.

This is shown by the following observations:—(1) One of the commonest agents employed in organic chemistry for the purpose of hydrolysing a substance is boiling with a dilute mineral acid, or subjecting in closed vessels to the action of superheated steam. On submitting proteids to the prolonged action of these reagents, products closely resembling or identical with those produced by the action of the proteolytic enzymes are obtained. (2) A small but decided increase in weight has been observed in the formation of peptone from proteid.[1] (3) Peptones can be converted artificially back into proteids by the use of reagents which are essentially dehydrating in their action. If fibrin-peptone be heated for an hour with acetic anhydride, the excess of anydride distilled off along with acetic acid formed in the process, and the residue treated with hot water, the greater part of it dissolves. When this solution is dialysed, there remains behind in the dialyser a solution which coagulates on boiling, and is precipitable by nitric acid or potassium ferrocyanide. Also, peptone heated for some time to 140° C. yields a substance which on solution in water shows more of the properties of a native albumin than of a peptone.[2]

Other theories regarding the digestion of proteids are—(1) That the proteids are polymers of the peptones, and that the process of digestion is a process of depolymerisation,[3] (2) that proteids and peptones are simply different isomeric forms of the same substance, and (3) the micellar theory,[4] according to which the proteids are composed of micelli, which are a kind of second order of molecule much more complex in structure. On peptonisation, the proteid first breaks up into its constituent micelli, then the micelli fall into molecules, in the chemical sense of the word, and these molecules are the peptone molecules.

[1] A. Danilewski, *Centralbl. f. d. med. Wissensch.*, Berlin, 1880, No. 42, S. 769.

[2] Henninger, *Compt. rend. Acad. d. sc.*, Paris, 1878, tome lxxxvi. p. 1464; Hofmeister, *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 206; Neumeister, *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 394.

[3] Maly, *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. ix. S. 585; *ibid.*, 1879, Bd. xx. S. 315; Herth, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. i. S. 277; *Monatsh. f. Chem.*, Wien, Bd. v.; Poehl, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1881, S. 1355; 1883, S. 1152; Loew, *Arch. f. d. ges. Physiol.*, Bonn, 1883, Bd. xxxi. S. 393.

[4] Griessmayer, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xiv. S. 26.

Ultimate chemical analysis shows that the composition of the peptones and albumoses is practically the same as that of the proteids from which they are formed; in some cases the proteids show a somewhat higher percentage of carbon and lower of hydrogen and oxygen than the proteids of their digestion, in others the reverse, but in no cases any very considerable variation. So that, if the process of peptonisation is one of hydrolysis, the peptone molecule must be out of all proportion greater than that of the molecule of water. Nevertheless the hydrolytic theory is the one most generally received, and against this unfavourable argument from analytical results must be set the other experiments already quoted.

The main differences between the proteids and peptones of physiological importance are the physical ones, that the latter are much more soluble, and are diffusible, though with difficulty, through membranes. It is indeed purely by physical means that we at present differentiate proteids and peptones and the intermediate products between them, and not by any well-marked chemical differences shown by them. The different proteids, albumoses, and peptones are classified and marked off from one another almost entirely by the behaviour of their solutions towards solutions of neutral salts of different strength, according to whether these dissolve or precipitate them. It is questionable whether it is justifiable on such a slender basis to assume, as is commonly done that these precipitates correspond to pure compounds. It ought to be remembered that these names at present only apply to certain precipitates, and that it is not at all known whether these represent distinct chemical substances, nor indeed what they do represent. Still less right have we to assume from mere proteid analyses that the products of digestion of different proteids yield substances distinctive of them and worthy of distinctive names.

The two proteolytic enzymes, pepsin and trypsin, closely resemble each other in their action on proteids, a series of very similar products being in each case evolved, which, generally speaking, become more soluble and probably simpler in constitution as the end of the process is approached. Still there is sufficient difference to warrant a separate consideration of the two processes.

Peptic Digestion of Proteids.

The stomach was recognised even by the ancients as a digestive organ, and its action attributed in many cases to the "animal heat" assisted by mechanical force. Digestion seems to have been first considered as a similar process to fermentation by van Helmont, and this view was also maintained by Sylvius.

Réaumur[1] seems to have been the first to experiment on gastric digestion. He carried out his successful experiments on a tame buzzard, which, like some other birds of prey, regurgitates after a time the more indigestible portions of its food. He administered various kinds of food, enclosed in small metallic tubes closed at one end and covered by muslin at the other, so as to prevent any mechanical action of the gizzard and yet allow the gastric juice to act; he found that meat was digested in the course of some hours, and in a shorter period was digested partially on the outside while the interior still remained untouched. Réaumur also obtained gastric juice by enclosing pieces of sponge in

[1] "Hist. Acad. roy. d. sc. de Paris," 1752, pp. 266, 461.

such tubes, but could not get it to act outside the body. Similar experiments were carried out by Stevens[1] of Edinburgh, who availed himself of the services of a juggler possessing a trick of swallowing stones and regurgitating them. This man he gave to swallow some hollow silver balls which were perforated with holes; the balls were screwed together in two halves and could be filled with meat. He found that the meat was rapidly dissolved and disappeared. To Stevens also belongs the credit of being the first to observe digestion outside the body. He obtained gastric juice from a dog's stomach, and found that when a piece of meat was subjected to its action in a warm place it became dissolved in about eight hours.

Soon afterwards Spallanzani confirmed these experiments, and showed conclusively that, under favourable conditions, the juice acted outside the body, and also that it had a marked action in preventing putrefaction.

Between 1825 and 1833 Beaumont published his classical observations on Alexis St. Martin. In 1834, Eberle[2] discovered a method of preparing an artificial gastric juice, which possessed all the digestive properties of the normal secretion, by acting on the gastric mucous membrane with dilute hydrochloric acid. Schwann[3] in 1836 gave the name pepsin to the active principle to which he supposed the gastric juice owed its activity.

Products of peptic digestion.—The first exact investigations into the nature of the products of gastric digestion are those of Meissner[4] and his pupils. After digestion in acid solution and filtration, a precipitate was obtained on nearly neutralising, to which the name of *parapeptone* was given.

There is a considerable difference of opinion among various authors as to what this parapeptone of Meissner is represented by in our more modern nomenclature. By some it is stated to have been syntonin. If Meissner had used a strongly peptic digestive medium, filtered and neutralised, just after the bulk of the proteid was dissolved, he would undoubtedly have obtained syntonin or acid albumin; but from his description it is evident that he was dealing with a substance afterwards discovered by Kühne, and renamed antialbumate. This substance seems by its behaviour to be indeed a close ally of acid albumin, and is obtained most readily by a more prolonged action of dilute acids at 40° C. than is necessary to form acid albumin. It is also formed to a small extent in a *weak* peptic digestive medium, probably from a similar cause. Like acid (or alkali) albumin, it is insoluble in water, but easily soluble in even very dilute acids or alkalies; but it differs from acid albumin in that when once formed it is not attacked by any pepsin in acid solution by which acid albumin is actively peptonised. It is, however, convertible into peptone (antipeptone) by the action of pancreatic juice, no leucine or tyrosine being simultaneously formed. Meissner was undoubtedly using very weak solutions of pepsin, and the action he obtained approximated to the prolonged action of weak acids alone at 40° C. The action of the pepsin present was too weak to catch, as it were, all the acid albumin on its way into antialbumate and peptonise it; and when once any antialbumate was formed, it could not then be attacked and peptonised. Meissner's product was thus almost purely anti-

[1] "De alimentorum concoctione," Edin., 1777.

[2] "Physiol. d. Verdauung nach Versuch.," Würzburg, 1834.

[3] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1836, S. 90.

[4] *Ztschr. f. rat. Med.*, 1859–1862, Dritte Reihe, Bd. vii. S. 1; viii. S. 280; x. S. 1; xii. S. 46; xiv. S. 303. Reviewed in *Biol. Centralbl.*, Erlangen, 1884, Bd. iv. S. 407.

albumate. If he had used a *slightly* stronger solution for a somewhat shorter time, he would have obtained a mixture which would have been partially peptonised and partially remained unchanged when subjected to the action afterwards of strong fresh pepsin and acid; if he had used a strongly peptic solution for a much shorter time, the result would have been purely acid albumin and no antialbumate whatever; giving with fresh pepsin, or more prolonged action, complete peptonisation.

On the addition of acid to the almost neutral *faintly* acid solution, a further precipitate formed, which Meissner regarded as a different substance, and called *metapeptone*. It was insoluble in very dilute acids (0·1 per cent.), soluble in stronger acids. A third residue obtained in the digestion of casein or fibrin he called *dyspeptone*; this was insoluble in dilute acids (2 per cent. HCl), but soluble in dilute alkalies and in stronger acids. This substance was probably a mixture of nucleins, with the substance subsequently described by Kühne as antialbumid.[1]

After the removal of these neutralisation products, various other substances were still left in solution; these Meissner classed together as *peptones*, distinguishing—

α-peptone, precipitable by concentrated nitric acid, as well as by potassium ferrocyanide and dilute acetic acid.

β-peptone, not precipitated by nitric acid, but by potassium ferrocyanide and strong acetic acid.

γ-peptone, not precipitated either by nitric acid or by potassium ferrocyanide and acetic acid.

Of these three substances only γ-peptone corresponds to the present-day definition of a peptone; the others were probably different albumoses.

A valuable side-light was thrown on the digestion products of proteids by Schützenberger's[2] researches on the prolonged action of acids and alkalies at high temperatures on these substances. It has already been indicated that peptonisation is the result obtained, followed finally by a splitting up into amido-acids.

Superheated steam possesses a similar peptonising action on proteids, and yields by prolonged action the usual amido-acids.[3] According to Neumeister,[4] the intermediate substances produced are, however, somewhat different, the substance first formed lies intermediate between the coagulable proteids and the albumoses. It is not coagulated by boiling, but in its behaviour towards the usual precipitants behaves like a coagulable proteid; this substance is termed *atmidalbumin*. By further hydration it yields a true albumose, which, however, differs somewhat in its properties from any of the albumoses naturally formed in digestion, and has been named *atmidalbumose*. Both atmidalbumin and atmidalbumose are precipitated by dilute acids, and are converted by boiling with dilute sulphuric acid into deutero-albumose. Similar products are produced by the action of the vegetable digestive ferment papoyotin or papain, and are in the end, by the prolonged action of this ferment, converted into amido-acids.[5]

[1] See p. 406.

[2] *Bull. Soc. chim.*, Paris, 1875, tome xxiii. pp. 161, 193, 216, 242, 385, 433; xxiv. pp. 2, 145; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1875, Bd. v. S. 299. Schützenberger's researches are referred to at length in the article on the "Chemical Constituents of the Body," pp. 30–32 of this volume.

[3] Lubavin, *Hoppe-Seyler's Med.-chem. Untersuch.*, Berlin, 1871, S. 480; Krukenberg, *Sitzungsb. d. Jenaisch. Gesellsch. f. Med. u. Naturw.*, 1886.

[4] *Ztschr. f. Biol.* München, 1890, Bd. xxvi. S. 57.

[5] Sidney Martin, *Journ. Physiol.*, Cambridge and London, 1885, vol. vi. p. 336.

Meissner's views as to the decomposition of proteids on digestion did not at first obtain much credence. The formation of a substance precipitated by neutralisation, and *incapable of further conversion by pepsin and an acid* in the course of normal digestion, was denied, and with right, by Brücke and others.

Brücke[1] stated that there was no such decomposition of the proteid molecule as Meissner indicated, but that fibrin is first dissolved and afterwards converted in great part into acid albumin, accompanied even at first by peptone in small quantity. If neutralisation takes place at this stage, a heavy precipitation is the result, and there remains in solution a small quantity of coagulable proteid (formed by the solution of the fibrin and not yet converted into acid albumin by the acid) mixed with albumoses and peptone. If, however, peptic digestion be allowed to proceed to completion, no precipitation occurs on neutralising, and the solution contains only albumoses and peptones. This shows that Meissner's parapeptone, as well as Kühne's antialbumate and antialbumid, which will be described later,[2] are not formed to any extent in active peptic digestion, but are merely products of prolonged action of dilute acid.

In order to study the products formed in peptic digestion, it is necessary to proceed with a digestive fluid which has been purified from products of digestion, due to self-digestion or otherwise, by one of the methods already described,[3] or else to take advantage of a peculiar property possessed by fibrin, and in a lesser degree by some other forms of proteid, of absorbing pepsin from solution.[4]

Any digestive fluid containing pepsin (such as that obtained by autodigestion of pig's gastric mucous membrane in dilute hydrochloric acid) is carefully neutralised, using powdered chalk for the purpose, so as to avoid all danger of alkalinity, by which the pepsin would be rapidly destroyed.[5] After neutralising and filtering, the fluid is shaken up with flakes of fibrin for some time; this is best done by blowing a stream of air through the mixture, placed in a tall vessel, by means of a Bunsen filter pump. In about an hour the fibrin becomes impregnated with pepsin, which, however, cannot attack it in the neutral fluid. So firmly adherent is the enzyme to the fibrin, that the latter may be freely washed without parting from it. If this fibrin be now placed in dilute hydrochloric acid (·2 per cent.) at 40° C., it is quickly dissolved and digested. Instead of neutralising the impure digestive fluid, it may be saturated with sodium chloride, which stops the digestive action of the pepsin; on now agitating thoroughly for about an hour, the fibrin is saturated with pepsin, after which it may be washed as before. This peculiar power of absorbing pepsin is shown in a varying degree by all solid forms of proteid. Fibrin possesses it most markedly, muscle fibre and casein also show it well, but coagulated proteids show it comparatively much more feebly.[6]

Fibrin, or other solid proteid, on digestion, swells up, dissolves, and is converted into syntonin or acid albumin. The same result is obtained

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1859, Bd. xxxvii. S. 131; 1861, Bd. xliii. S. 601.

[2] See pp. 406–409. [3] See p. 402.

[4] Von Wittich, *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 443; K. Mann, "Ueber die Absorption der proteolytischen Enzyme durch die Eiweisskörper," Inaug. Diss., Würzburg, 1892, S. 23.

[5] Langley, *Journ. Physiol.*, Cambridge and London, 1882, vol. iii. p. 253.

[6] Wurtz, *Compt. rend. Acad. d. sc.*, Paris, 1881, tome xciii. p. 1104; A. Fick, *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1889, S. 23; K. Mann, Inaug. Diss., Würzburg, 1892.

with acid alone, but incomparably more slowly. The acid and ferment seem to mutually assist each other. Pepsin alone is inactive, the acid alone acts with extreme slowness, but in the presence of the acid the ferment speedily dissolves the proteid, which is then rapidly attacked by the acid and converted into acid albumin.

When the proteid undergoing digestion is fresh fibrin which has not been previously subjected to heat coagulation, a body possessing the properties of a globulin is found in the solution in the first stage of digestion, before or just when complete solution has taken place; a similar body is also said to be formed in small quantity as a first product of digestion of other forms of proteid.[1] In a recent paper it is stated by Arthus and Huber[2] that this globulin is simply dissolved fibrin. These authors found such a body coagulating at 56° C. on digesting unboiled fibrin; but boiled fibrin yielded no such product. They also determined that "Witte's Peptone" dissolved unboiled fibrin, at 40° C., giving a solution which coagulated on heating at 56°, 68°, and 75° C.

The acid albumin is next attacked by the pepsin and further altered, giving rise to a number of substances called *albumoses*, *proteoses*, or *propeptones*, and these in turn are slowly and incompletely converted into peptones. Here the action of pepsin ceases.

Cleavage theory of proteid digestion.—The cleavage theory of proteid digestion was first enunciated by Kühne in 1877.[3] He describes the digestion of albumins by trypsin as taking place in two stages: in the first stage the albumin is changed into peptone (amphopeptone); in the second stage, one-half of this peptone (hemipeptone) is further changed, while the other half (antipeptone) remains unaltered. Peptic digestion is not essentially different from the first stage of tryptic, and while it is not possible to obtain two bodies from pepsin peptone, still it is probable that this substance is a mixture of two bodies, antipeptone and hemipeptone, as is also the case after the first stage of tryptic digestion.

Unable to isolate two bodies from the end products of peptic digestion, one of which should remain unchanged when subjected to tryptic digestion, while the other broke up under like treatment into leucine and tyrosine, Kühne surmised that the cleavage might take place earlier in the process of peptic digestion, and that more success might attend an attempt to separate the precursors of anti- and hemipeptone, namely, the corresponding albumoses, by interrupting peptic digestion at an early stage, and experimenting upon the products then in solution. By interrupting peptic digestion at an early stage, two substances were obtained: one was a neutralisation precipitate, which, on tryptic digestion, afterwards yielded only antipeptone, and was hence named *antialbumose*; the other, obtained from the filtrate, was decomposed by trypsin, with formation of leucine and tryosine, and was hence named *hemialbumose*.[4] Kühne[5] also reinvestigated the action of acids, renaming Schützenberger's hemiprotein *antialbumid*, and a body

[1] Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1859, Bd. xxxvii. S. 182; Otto, *Ztschr. f. physiol. Chem.*, Strassburg, 1883, Bd. viii. S. 129; Hasebroek, *ibid.*, 1887, Bd. xi. S. 348; A. Herrmann, *ibid.*, 1887, Bd. xi. S. 508; Neumeister, *Ztschr. f. Biol.*, München, 1890, Bd. xxvii. S. 310.

[2] *Arch. de physiol. norm. et path.*, Paris, 1893, tome xxv. p. 447.

[3] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1877, N. F., Bd. i. S. 236.

[4] *Vide infra.*

[5] *Loc. cit.*

closely resembling Meissner's parapeptone *antialbumate*; considering them anti bodies, from the fact that they do not yield leucine and tyrosine on tryptic digestion, but are, though with difficulty (especially in the case of antialbumid), converted into antipeptone.

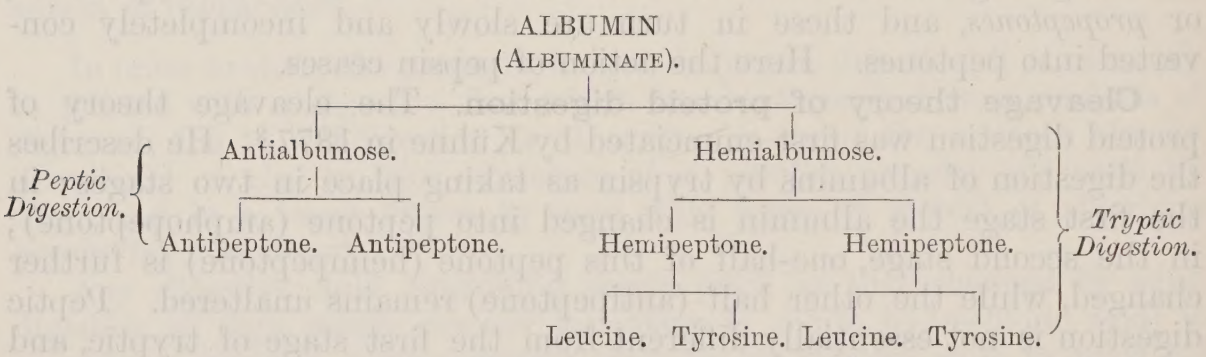
Kühne gave the following graphic representations of the cleavage of proteids by acids and by digestion :—

Scheme of Proteid Cleavage by Acids.

ALBUMIN.

Antigroup.	Hemigroup.
Antialbumid.	...
Antialbumate.	...
Antialbumose.	Hemialbumose.
Antipeptone.	Hemipeptone.

Scheme of Digestive Cleavage of Proteids.



Kühne, in conjunction with Chittenden,[1] subsequently investigated more minutely the intermediate products in peptic digestion, and those formed by the action of dilute acids. The following is an account of the substances obtained and their mode of preparation : —

Antialbumid.—This substance was prepared as follows :—The white of fifty eggs, freed from membrane and much diluted, was made feebly acid with sulphuric acid and coagulated by boiling. The coagulum was suspended in 1300 c.c. of water containing 7 c.c. of sulphuric acid and heated to 100° C. ; after ten hours it appeared little altered and was filtered off. The filtrate gave on neutralisation a precipitate principally composed of acid albumin. After removal of the first acid, the albumin which had remained undissolved was heated with 3 litres of $\frac{1}{2}$ per cent. sulphuric acid to 100° C. for nineteen hours, then collected on a filter and completely washed.

The albumid thus obtained was insoluble both in dilute and concentrated acetic acid, and in hydrochloric acid of 1·4 per mille and stronger, but easily soluble in dilute caustic soda solution and in dilute alkaline carbonates, from which it was precipitated by concentrated sodium chloride. Purified by digestion with gastric extract and 4 per mille hydrochloric acid for six hours at 40° C., it remained undissolved, but changed in appearance, becoming clotlike. The clot was washed with water, dissolved in 1 per cent. solution of sodium carbonate, filtered, reprecipitated with sulphuric acid, and washed again. It now dissolved in 2 per mille hydrochloric acid, and in this solution was digested with good peptic extract for eighteen hours. It was unchanged, and reappeared, in equal amount to the eye, on neutralisation of the solution.

[1] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 159.

Now washed with water until no reaction for chlorides was obtained, and afterwards treated with alcohol and ether, it formed a powder of slightly yellowish colour. A part of this purified antialbumid was dissolved in sodium carbonate solution of ·5 per cent., and treated with a dialysed and very active trypsin solution at 37°–38° C. After thirty minutes the mixture began to be turbid, and in two hours solidified to a clot. By breaking up the clot and filtering, the fluid part was separated from the clot; it was made scarcely turbid by neutralisation, and yielded by further digestion no new precipitate, but contained a fair amount of peptone.

The separated clot was soluble in hydrochloric acid of 2 per mille, but as insoluble in sulphuric acid of 4 per mille as the original precipitate. It was completely precipitated from solution in 1 per cent. sodium carbonate solution by concentrated sodium chloride solution. From this it seems that in the coagulation of albumid in trypsin no change takes place other that its becoming more insoluble in sodium carbonate solution. The action of trypsin in more alkaline solution was next tried; after the first precipitation of the albumid it was dissolved in ·75 per cent. sodium carbonate solution, digested with dialysed tryptic fluid, neutralised and filtered. The clotlike albumid was dissolved *in* 5 *per cent. sodium carbonate solution*, and by repeated digestion with dialysed tryptic fluid, *the greater portion* was converted into antipeptone. The final residue from this much accentuated tryptic digestion was completely insoluble even in 5 per cent. sodium carbonate solution, but dissolved in 1 per cent. caustic soda. Precipitated by neutralising with hydrochloric acid, washed, redissolved in sodium carbonate, and treated with trypsin anew, it was again completely thrown out as a clotlike coagulation. No leucine or tyrosine was present in the tryptic filtrates.

How widely this account differs from the statement which occurs in most text-books, that antialbumid is not attacked by pepsin, but is converted into antipeptone by trypsin, may easily be seen. In a fluid of equal alkalinity to that found in the body, antialbumid is no more digested by trypsin than it is by pepsin and hydrochloric acid. Now it has been shown that trypsin is most active in a sodium carbonate solution of about 1 per cent.,[1] and considerably less active in one of 5 per cent.; why then does trypsin in 5 per cent. solution do that which it is unable to do in 1 per cent. solution? Obviously because the 5 per cent. solution dissolves the clot of antialbumid, while the 1 per cent. solution does not. In the former case a weaker trypsin acts on antialbumid in solution; in the latter, a stronger trypsin on antialbumid as an insoluble precipitate.

Be this as it may, antialbumid is only with great difficulty and incompletely peptonised by trypsin. In all its properties, from its mode of formation onward, the substance appears to be merely a very insoluble form of acid albumin.

Antialbumose.—By a fractionated peptic digestion, Kühne and Chittenden[2] obtained a substance which they termed antialbumose. The preparation of this substance from white of egg is as follows:—

The white of fifty eggs was freed from membrane, diluted and coagulated by boiling after acidifying with acetic acid. The coagulated proteid was digested in two litres of 4 per mille hydrochloric acid and one litre of dialysed gastric extract for one and a half hours at 40° C., it was then allowed to cool to the temperature of the room and filtered from the undissolved part, *the process of filtration occupying two days.* The undissolved residue was again treated with fresh gastric extract until it was all dissolved, which occupied

[1] See p. 338.

[2] *Loc. cit.*, p. 171.

fifteen hours. After filtration the fluid was neutralised, and the neutralisation precipitate separated. This precipitate was digested anew for forty-eight hours with 150 c.c. of strong gastric extract, after which it was re-obtained on neutralisation not sensibly diminished in amount. Dissolved in ·75 per cent. sodium carbonate, and mixed with powerful dialysed pancreatic extract, it gave no clot (see "Antialbumid") when kept for forty-eight hours at 40° C., and neutralisation precipitated only a part, the rest being converted into peptone. This last neutralisation precipitate showed the properties of antialbumid; dissolved in sodium carbonate solution of 1·8 per cent., it was clear at first, but began to cloud in one to two hours, and in twenty-four hours about half had set into a thick clot which could not be peptonised completely by either peptic or tryptic digestion. The various pancreatic solutions separated from the neutralisation precipitates contained only peptone, and were free from leucine and tyrosine. The antipeptone here obtained contained 30 per cent. of ash.

Of these three anti-compounds it is only claimed that one, antialbumose, is a product of natural digestion; the other two, antialbumid and antialbumate, are admittedly products of acid action or of acid and *very weak* peptic solution, which amounts to the same thing,—and the fact that they cannot be converted into peptones by the prolonged and repeated action of pepsin and hydrochloric acid proves that they are not natural products of strong peptic digestion in which no such inconvertible residue is formed. Antialbumose is commonly stated to be convertible by prolonged peptic digestion into peptone; but, as may be seen from the above description, it is not materially altered by forty-eight hours' digestion with a strong extract of gastric mucous membrane, and even with trypsin a considerable portion is left unaltered, betraying all the properties of antialbumid. Antialbumose possesses all the chemical properties of an acid albumin and none of those of the albumose class, so that its name is a misnomer; no such substance as an antialbumose has actually been isolated. Antialbumid, antialbumate, and antialbumose, to place them in the order of their solubility and facility for undergoing decomposition, are three substances all of which are remarkably resistant to both peptic and tryptic digestion, and belong more to the class of acid albumins than to any other. It is now generally recognised that acid albumin is a generic and not a specific term, and it is to be hoped that room will soon be found for these three bodies in this class, and the terminology of digestion left a little less complicated than it is at present.

It may be asked, Why was antialbumose, if it is not a natural product of peptic digestion, obtained in the above experiment? The authors themselves remark on the close resemblance between their product and Meissner's parapeptone. The latter is produced either by the action of dilute acid or of a very weak pepsin solution in the presence of acid. Now for two days, while filtering at atmospheric temperature, after the first hour and a half of digestion, the substance was under exactly the proper conditions for the production of parapeptone. Finally, no product so resistant to both pepsin and trypsin, as this substance is shown to be by the above description, is formed during uninterrupted digestion.

Another method for preparing "antialbumose."—Kühne and Chittenden[1] also prepared antialbumose from fibrin by a somewhat similar course of procedure, except that there was here no two days' delay in filtering, since the fibrin was more quickly dissolved. There is, however, an objection no less fatal, as will be pointed out after a description of the process.

[1] *Loc. cit.*

Five hundred grms. of unboiled fibrin, squeezed as dry as possible with the hand, were placed at room temperature for twenty-four hours in 5 litres of 0·2 per cent. hydrochloric acid; the mixture was then heated to 37° C., and 100 c.c. of gastric extract added. Solution took place inside an hour, after which the fluid was filtered through a hair sieve, digestion stopped by neutralisation, and the neutralisation precipitate filtered off. This precipitate is stated to be essentially antialbumose. It was long washed with water, *and did not then dissolve easily in* 0·2 *per cent. hydrochloric acid, so was heated for some hours at* 40° C. This acid solution was treated with an equal volume of strong gastric extract in 0·2 per cent. hydrochloric acid for forty-eight hours, *again a heavy neutralisation precipitate* was obtained. This precipitate, after washing with water thoroughly till no biuret peptone reaction was given, was treated with sodium carbonate solution of 2·5 *per cent., in which it was not easily soluble,* and the solution was not clear until it had been digested for forty-eight hours at 48° C. with trypsin. Even then, on neutralising, a precipitate behaving like antialbumid was obtained. Redissolved in 2·5 per cent. sodium carbonate solution, and redigested with trypsin, it was again precipitated in clotlike flakes, and was only very slowly and partially converted by repeated tryptic digestion.

Here, again, there is no guarantee, after heating the first neutralisation precipitate for some hours with 0·2 per cent. hydrochloric acid in order to dissolve it, that a natural digestion product remains to be dealt with in the subsequent processes. In addition, the obstinate resistance of the substance to both peptic and tryptic digestion proclaims it a product of experimental procedure, and not a true stage in natural or uninterrupted digestion.

Hemialbumose.—Kühne and Chittenden[1] also obtained a precipitate, to which they gave the name of hemialbumose; this was obtained from the products of fractional peptic digestion in the filtrate after the removal of the so-called antialbumose by neutralisation. This filtrate was concentrated to one-fourth of its volume, acidified with acetic acid, boiled and filtered from a scanty coagulum, again concentrated and precipitated by the addition of excess of alcohol. In this precipitate by alcohol, the authors recognised, besides peptones, two forms of albumose, soluble and insoluble hemialbumose. The precipitate was rubbed up with cold water, until the wash water no longer gave the biuret reaction. A part of the albumose (soluble hemialbumose) went into solution, accompanied by all the peptone, a part remained insoluble (insoluble hemialbumose). The latter substance was not pure, but contained a proteid substance insoluble in 2 per cent. acetic acid and in sulphuric acid of 0·4 per cent., and with difficulty soluble in dilute caustic soda solution. The "insoluble hemialbumose" was separated from this by treating with boiling water. From solution in boiling water a part of the "insoluble hemialbumose" was precipitated as the solution cooled. This was separated; the remainder was precipitated from the cold solution and added to it. The "soluble hemialbumose" was obtained, free from its admixture with peptone in the cold water extract, by Salkowski's method of boiling with excess of sodium chloride and dilute acetic acid so as to form a saturated solution, washing the precipitate with saturated sodium chloride solution, dissolving in water and dialysing until the dialysate gave no reaction for chlorides with silver nitrate.

These hemialbumoses on tryptic digestion yielded leucine and tyrosine abundantly, but could not be completely broken up by such digestion, a variable amount of peptone being always left, no matter how prolonged the digestion, which could only (on the cleavage theory) be antipeptone, and so pointed to impurities in the form of anti-compounds in these

[1] *Loc. cit.*

hemialbumoses (or otherwise to the non-existence of cleavage at the albumose stage into hemi and anti groups). Nor, when these hemialbumoses were subjected to more prolonged digestion yielding hemipeptone (?), could this substance be completely broken up by prolonged tryptic digestion.

Kühne[1] also described as hemialbumose a substance occasionally found in the urine of patients suffering from osteomalacia, and first discovered by Bence Jones. Much has been made of the importance of this albumose by the supporters of the cleavage theory, but there is no more evidence that it is a pure hemialbumose than there is in the case of the substances described above; that is to say, it has not been shown to be completely broken up by tryptic digestion, and this is the crux of the whole question. The fact that it yields leucine and tyrosine proves nothing. It has not been experimentally shown that no peptone is left after the prolonged action of trypsin upon it.

Separation of the various albumoses from the "hemialbumose" precipitate.—Stimulated by a desire to obtain a pure hemialbumose which should be capable of complete decomposition past the peptone stage by trypsin, and encouraged in the belief that hemialbumose was a mixture, as well by the known existence of two physically different forms (the soluble and insoluble described above) as by certain inconstancies in its behaviour towards sodium chloride, Kühne and Chittenden[2] set to work again upon the subject, and although they did not quite achieve their object, produced a research which, whether the cleavage theory stands or falls, must, from the experimental point of view, always remain of the highest value, containing as it does the first basis for a classification of the albumoses, the first light cast upon the relationship of this class of proteids.

From the hemialbumose described in their previous paper, they were able to separate, by the action of sodium chloride under various conditions, four substances with the following properties:—

1. *Protoalbumose.*—Precipitated by saturation with sodium chloride, soluble in cold and hot water.

2. *Heteroalbumose.*—Also precipitated by saturation with sodium chloride, but insoluble in cold and in boiling water; soluble in dilute and in moderately concentrated saline solution.

3. *Dysalbumose.*—The same as heteroalbumose, but insoluble in saline solution. This solution was recognised to be merely a more insoluble modification of heteroalbumose; each of the two substances is easily convertible into the other. Dysalbumose corresponds to the "insoluble albumose" of the earlier paper.

4. *Deuteroalbumose* is not precipitated by saturation with sodium chloride alone, but is precipitated by saturation with sodium chloride in the presence of acetic acid, and is soluble in water.

These various albumoses were subjected to tryptic digestion, and it was found that none was a pure hemialbumose,—all yielded more or less unconvertible peptone accompanied by leucine and tyrosine. A bigger yield of amido-acids was obtained from protoalbumose and deuteroalbumose than from heteroalbumose; indeed, the latter showed itself to be more an anti- than a hemi- body, while protoalbumose yielded very little peptone and an abundance of amido-acids. After this evidence

[1] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 209.
[2] *Ibid.*, 1884, Bd. xx. S. 11.

the term hemialbumose, as applied to the substance, or rather mixture of substances, described above, ought to have speedily disappeared; unfortunately it has not yet done so.

Soon after this a valuable aid to the study of the albumoses was found in the discovery of Wenz,[1] that saturation with ammonium sulphate precipitated all albumoses from solution, while the peptones remained dissolved. Heynsius[2] first noticed the powerful action of ammonium sulphate as a proteid precipitant, but fell into error in thinking that it precipitated peptones as well. More careful experiments by Wenz, in Kühne's laboratory, showed that it did not precipitate peptones, and so it was instituted as a means of separating albumoses and peptones. The statement, however, that saturation with ammonium sulphate totally precipitates albumoses and leaves peptones dissolved, can only be made with a certain reservation. Certain proteid substances remain unprecipitated by saturation with ammonium sulphate, and these may conventionally be labelled peptones; but it has been shown[3] that, in order to precipitate completely bodies which had been known as albumoses before the introduction of ammonium sulphate, it is necessary to help the ammonium sulphate by saturating in dilute solution and with varying reaction. If these bodies had not been classed with the albumoses before Wenz's discovery, they would probably now be peptones; so conventional and artificial as this is the proteid classification with which at present we are forced to be content. In little or nothing except unimportant physical differences are the albumoses and peptones distinct. If ammonium sulphate did not exist, it would be difficult to say how to draw a sharp line between them;[4] both classes of bodies give the same reaction to the biuret test, and both are diffusible, though the albumoses more slowly so than the peptones.[5]

Separation of albumoses and peptones.—The following is the method recommended by Kühne[6] for separating albumoses from peptones:—

> The fluid containing the products of digestion is freed from albuminates and coagulable proteids in the usual manner, and then, when *sufficiently diluted* and of nearly neutral reaction, is saturated while boiling with ammonium sulphate, and separated on cooling from the excess of salt and precipitated albumose. The solution is again heated, and after it commences to boil it is made strongly alkaline by the addition of ammonia and ammonium carbonate, then again saturated with ammonium sulphate, and once more allowed to cool, when a second precipitation of albumose and excess of salt takes place. A third time heated, until the smell of ammonia disappears, it is once more saturated while warm and made decidedly acid in reaction by the addition of acetic acid, when, on cooling, a third and last precipitation of albumose takes place, and the filtered fluid is supposed to contain nothing proteid except peptone; amphopeptone if the original fluid was the result of gastric digestion. The albumoses can be obtained by dialysis and concentration from the united precipitates.

[1] *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 1.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiv. S. 330.

[3] Kühne, *Ztschr. f. Biol.*, München, 1893, Bd. xxix.

[4] For a discussion of this point see Pekelharing, *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxii. S. 185; 1881, Bd. xxvi. S. 515; *Internat. Beitr. z. wissensch. Med. Festschr. R. Virchow . . .*, Berlin; *Ztschr. f. Biol.*, München, 1891, Bd. xxviii. S. 567; Neumeister, *ibid.*, S. 361; Kühne, *ibid.*, S. 571.

[5] See Kühne, *Ztschr. f. Biol.*, München, 1892, Bd. xxix. S. 20; Chittenden and Amerman, *Journ. Physiol.*, Cambridge and London, 1893, vol. xiv. p. 483.

[6] *Loc. cit.*

The amphopeptone is obtained from the filtrate after removal of the ammonium sulphate by complicated methods, consisting essentially in the removal of ammonium sulphate as far as possible by concentration; solution of the amphopeptone in weak alcohol; removal of as much ammonium sulphate from the weak alcohol as possible by a freezing mixture; removal of the alcohol by distillation; removal of the last portions of ammonium sulphate by boiling with barium carbonate; removal of the last traces of barium salts by cautious addition of dilute sulphuric acid; and finally, precipitation of the amphopeptone by excess of absolute alcohol.

Neumeister's method for separating the albumoses of peptic digestion.—The method of Kühne and Chittenden for the separation of the various albumoses has been perfected by Neumeister,[1] who has in addition proved that these bodies are not formed synchronously in the process of digestion, or other form of hydrolysis, but that there are two stages in the process. In the first stage *proto-* and *hetero*albumoses are formed, which are called for this reason *primary* albumoses; in the second stage each of these primary albumoses gives rise to a deuteroalbumose, and these deuteroalbumoses are hence called *secondary* albumoses.

Since heteroalbumose is *completely* and protoalbumose only *partially* precipitated by saturation with sodium chloride in neutral solution, while deuteroalbumose is not precipitated at all, it is easy, from a mixture of all three albumoses, to obtain a solution containing only heteroalbumose and protoalbumose; and on dialysis of this solution heteroalbumose, being insoluble in water, is precipitated alone, leaving in solution only pure protoalbumose. In this way pure proto- and heteroalbumoses can be obtained, but the preparation of pure deutero-albumose is not quite so easy. In the filtrate from saturation with sodium chloride there is not only deuteroalbumose but the unprecipitated residue of the protoalbumose, and on adding acetic acid this is thrown out along with the deuteroalbumose. However, a loophole is left in the fact that just as saturation in neutral solution does not precipitate all the protoalbumose, so saturation in acid solution does not precipitate all the deuteroalbumose. Neumeister took advantage of this, sacrificed the first portion of deuteroalbumose thrown out by the acetic acid, accompanied by the last portions of protoalbumose, and then precipitated the fraction of deuteroalbumose left alone in solution by saturation with ammonium sulphate.

Kühne and Chittenden had already got round this difficulty of isolating deuteroalbumose by treating a dried mixture of the albumoses, such as is found in Witte's peptone, with neutral and saturated solution of sodium chloride. Here the deuteroalbumose only passes into solution. Although the proto-albumose would only be partially thrown out of solution by saturating with sodium chloride, yet it has not the power when dry to pass into solution in such a solvent. Witte's peptone is, however, a variable mixture, and Neumeister, working with other samples, was unable to reobtain Kühne and Chittenden's result; it may be that they were working with a sample containing little or no protoalbumose.

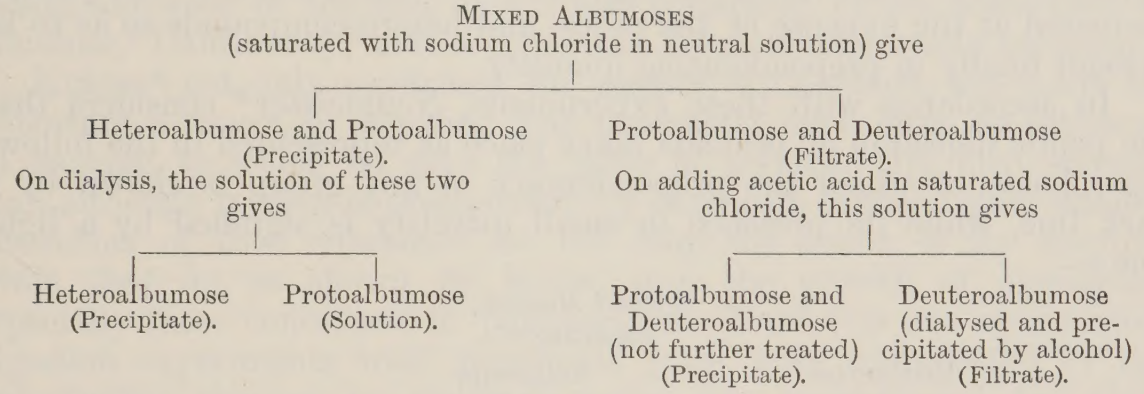
Neumeister effects the separation as follows:—

The faintly acid solution is saturated with ammonium sulphate, and so separated from peptones. The precipitate is dissolved by the addition of water, separated from the excess of the salt by dialysis, and then the neutral

[1] *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 381; *ibid.*, 1888, Bd. xxiv. S. 267; *ibid.*, 1890, Bd. xxvi. S. 324.

solution is precipitated by saturation with sodium chloride, so throwing out all the heteroalbumose and part of the protoalbumose which are separated by dialysis. In the filtrate from saturation with sodium chloride in neutral solution are the remainder of the protoalbumose and the whole of the deuteroalbumose; to this, acetic acid solution, which has been saturated with sodium chloride, is added, till a small portion filtered through a dry filter paper no longer gives a precipitate with copper sulphate solution.[1] The mixed precipitate of *proto-* and *deutero*albumoses is now filtered off, and deuteroalbumose is isolated from the filtrate by neutralising, dialysing off the sodium chloride, and precipitating by saturating with ammonium sulphate, or by adding excess of alcohol.

This method of separating the albumoses may be shown schematically thus :—



Neumeister also tested the action of hydrolysing agents on pure *proto-* and *hetero*albumoses prepared as has just been described. He showed that boiling for three-quarters of an hour with 5 per cent. sulphuric acid was sufficient to convert protoalbumose into deuteroalbumose accompanied by some peptone. This was shown by the absence of turbidity on dialysis after neutralising (absence of heteroalbumose), by saturation in neutral solution with sodium chloride causing no precipitate (absence of protoalbumose), and by precipitation occurring on making the saturated solution in sodium chloride acid with acetic acid (presence of deuteroalbumose). In a similar fashion the conversion of heteroalbumose into deuteroalbumose, by boiling with acid, was demonstrated; here a considerable formation of antialbumid was observed during the process. On peptic digestion *proto-* and *hetero*albumose each yielded a deuteroalbumose, but they behaved differently towards trypsin. In the case of heteroalbumose, a specific point could easily be determined, in the course of digestion with trypsin in 0·2 per cent. sodium carbonate solution, when, in the presence of a considerable quantity of peptone, only deuteroalbumose was present; on the other hand, deuteroalbumose could not be obtained in large quantity by the action of trypsin on protoalbumose; the products obtained were chiefly amido-acids accompanied by a little peptone, this being probably due to the ease with which the deuteroalbumose formed from protoalbumose undergoes decomposition.

Neumeister confirms the results obtained by Kühne and Chittenden, that heteroalbumose is principally an antialbumose, but has some hemialbumose mixed with it, while the composition of protoalbumose is the exact reverse, it being essentially a hemialbumose, always accompanied, however, by some antialbumose. The yield of unalterable peptone was, however, so small in some experiments as to induce Neumeister to believe that perfectly pure protoalbumose would contain only hemi groups, or, in other words, be completely convertible by tryptic digestion into amido-acids. The deuteroalbumose

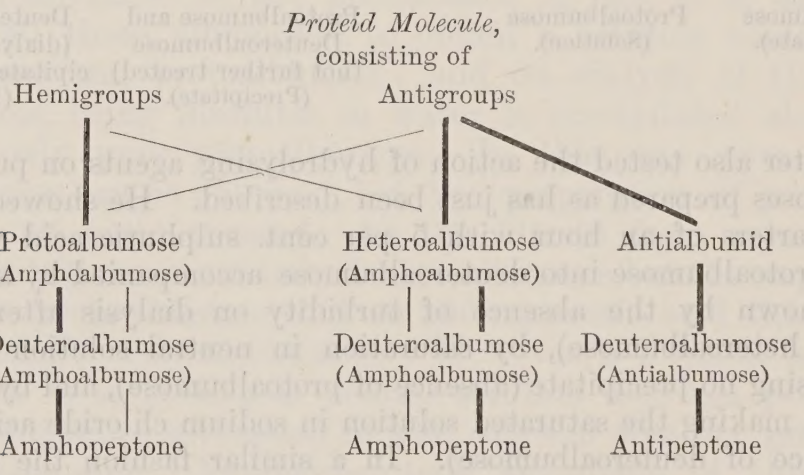
[1] This is a much more delicate test for protoalbumose (given by 1 in 5000) than acetic acid and saturated sodium chloride solution (1 in 2000).

formed from protoalbumose and that from heteroalbumose are distinct bodies, being distinguished by the fact that the deutero-compound formed from protoalbumose is to some extent soluble in saturated solution of ammonium sulphate.[1]

Starting with fibrin, and forming albumoses from it both by the action of acids and by peptic digestion, Neumeister also showed that in order of time *proto-* and *hetero*albumoses first appeared, to be followed later in the process by *deutero*albumose.

Fibrin was boiled for three-quarters of an hour with 1 per cent. sulphuric acid, after which the fluid was neutralised, and the neutralisation precipitate removed. In the filtrate both proto- and heteroalbumoses were found, but not a trace of deuteroalbumose; the latter first appeared after some hours' boiling, and by a continuance of the process increased at the expense of the proto- and hetero-compounds so as to be present finally in preponderating quantity.

In accordance with these experiments, Neumeister[2] considers that the peptic digestion of proteids takes place as represented in the following scheme, in which the preponderance of any group is shown by a dark line, while its presence in small quantity is signified by a light line:—



Tryptic Digestion of Proteids.

The proteolytic action of the pancreatic juice has not been known for nearly so long a period as that of the gastric juice; it was first clearly proclaimed by Corvisart[3] in 1857, although this author refers to an earlier statement, by Purkinje and Pappenheim in 1836, that extracts of pancreas possess a dissolving action on proteids.

Claude Bernard[4] knew that pancreatic juice in the presence of bile had the power of dissolving proteids, but stated that when alone it had no such action, unless the proteid matter had previously been subjected to the action of bile. This error was removed by Corvisart, who clearly proved that pancreatic juice alone at the temperature of the

[1] *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 267.

[2] *Ibid.*, München, 1887, Bd. xxiii. S. 381. See also Neumeister, "Lehrbuch der physiologischen Chemie," Jena, where Neumeister concludes: — "The expression hemipeptone has, according to this representation, only a theoretical meaning, and the term hemialbumose corresponds to older notions and ought to be allowed gradually to disappear from the text-books."

[3] "Collection de mémoires sur une fonction peu connue du pancréas, la digestion des aliments azotés," Paris, 1857.

[4] "Leçons de physiologie expérimentale," 1856, tome ii. p. 440.

body has a powerful digestive action on proteids in fluids of either alkaline, acid or neutral reaction. In addition, he showed that infusions of the fresh gland possess a similar action, that the active material is precipitated by excess of alcohol and is dissolved again on the addition of water to the precipitate, and that the activity of extracts of the gland depends on the time after a meal at which the animal is killed, being most active when a gland is extracted that has been obtained from an animal killed six to nine hours after a full meal. Corvisart also showed that the proteids are not merely dissolved, but converted into substances possessing the same general characters as those formed in peptic digestion.

These important results were denied at first by some observers, who failed for some reason to obtain them on repeating Corvisart's experiments, but were in the end abundantly confirmed by the researches of Meissner,[1] Danilewsky,[2] and Kühne,[3] and are now universally accepted.

Kühne[4] not only confirmed the results of Corvisart, but made an important advance, by showing that pancreatic juice owes its action to an enzyme, to which he gave the name of trypsin. He next showed that, although trypsin is precipitated by excess of salicylic acid, smaller quantities of that substance do not stop the action of the enzyme, while they do, as shown by Kolbe, stop the growth of organisms, especially those concerned in putrefaction. Until this was ascertained, digestion experiments with pancreatic juice were complicated by the putrefactive changes by which digestion was accompanied, for, while trypsin acts in a neutral, and even in a faintly acid medium, its action is stopped and the ferment gradually destroyed in a medium sufficiently acid to stop the growth of bacteria by virtue of its acidity alone, so that no one had been able to carry out prolonged experiments on pancreatic digestion without the accompaniment of putrefaction. For this reason it was unknown whether certain substances which appear towards the end of the digestion were really due to the action of the enzyme or were products of the putrefaction. Kühne was the first to carry out antiseptic digestion, and to show that these substances are really formed by the agency of the trypsin; he also perfected a method of freeing solutions of the enzyme from the products of proteid digestion, resulting either from the self-digestion of the gland in the preparation of the extracts or otherwise, thus clearing the way for a study of the various products formed by the action of trypsin on proteids.

Instead of preparing a purified solution of trypsin, which is a rather troublesome process, the power possessed by fibrin of absorbing the ferment, as described in the case of peptic digestion, may be utilised here also; but if the digestion of coagulated proteids is to be observed, a purified solution of trypsin must be first prepared.[5]

The first action of trypsin seems to be a simple solution of the proteid which is undergoing digestion. This effect is most easily observed if fibrin is the proteid undergoing digestion, when the coagulable proteid present in the solution, just before the fibrin is completely dissolved, has

[1] *Ztschr. f. rat. Med.*, 1859, Dritte Reihe, Bd. vii. S. 1.
[2] *Virchow's Archiv*, 1862, Bd. xxv. S. 279.
[3] *Ibid.*, 1867, Bd. xxxix. S. 130.
[4] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1877, N. F., Bd. i. S. 233.
[5] See Neumeister, "Lehrbuch der physiologischen Chemie," Jena, 1893, S. 198; K. Mann, "Ueber die Absorption der proteolytischen Enzyme durch die Eiweisskörper," Diss., Würzburg, 1892, S. 23.

the properties of a globulin, but in the case of serum albumin no such formation of a globulin takes place.[1] If the proteid employed has previously been coagulated, no formation of a coagulable proteid is observed, the first product being apparently deuteroalbumose.[2]

The appearances presented by proteid undergoing solution by the action of pepsin and of trypsin respectively, are characteristically different. In the case of pepsin and hydrochloric acid, the proteid swells up, becomes transparent or translucent, and gradually dissolves; while, by the action of trypsin in alkaline solutions, the proteid does not swell up or become clearer, but is attacked and eroded from the outside.

After being dissolved, the proteid is further attacked by the trypsin and decomposed into various products, the final result being a certain amount of peptone which is not further acted on, accompanied by various nitrogenous bodies, of which those occurring in largest quantity are two amido-acids, leucine and tyrosine.

The primary albumoses of peptic digestion are not found among the intermediate products of tryptic digestion. No matter at what stage digestion is interrupted, no trace of either proto- or heteroalbumose is found; the only albumose present is deuteroalbumose.[3]

Neumeister suggests that this may be due to the protoalbumose being broken up as rapidly as it is formed into amido-acids, while the heteroalbumose is immediately converted into deuteroalbumose. Be this as it may, the experimental fact is, that neither protoalbumose nor heteroalbumose are found at any stage of tryptic digestion.

According to Neumeister, the deuteroalbumose present is an anti-compound not yielding any amido-acids when subjected to the further action of trypsin.[4]

Peptone is formed much more rapidly in tryptic than in peptic digestion, the preliminary stages being apparently rushed through; while in peptic digestion scarcely any peptone is formed before complete conversion into albumoses has taken place, and complete peptonisation never occurs.

The most essential difference between the digestive action of trypsin and that of pepsin lies in the discovery of Kühne, that the action of the former enzyme does not cease with the formation of peptone, but that approximately one-half of the proteid, or of the peptone formed from it, is converted into a number of cystalline substances of much simpler composition.

Not only does this take place in the direct tryptic digestion of proteids, but if peptone formed by peptic digestion be submitted to tryptic digestion, about one-half of it is decomposed in the above fashion. This experiment led Kühne to the cleavage theory, and to naming, on the basis of this theory, the peptone of peptic digestion, *amphopeptone*; the peptone remaining after the completion of tryptic digestion, and which is no longer affected by renewed digestion, *anti*peptone; and that hypothetical substance which is supposed to form one moiety of the amphopeptone, and be broken up by the action of the trypsin,

[1] Neumeister, *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 398; *ibid.*, 1890, Bd. xxvii. S. 311; Herrmann, *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 521.

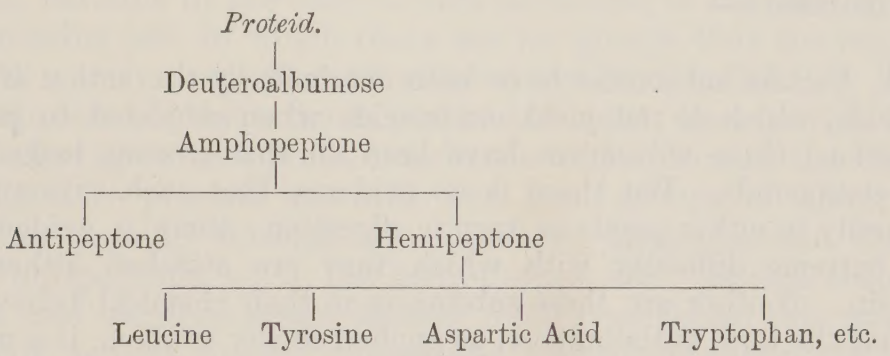
[2] When trypsin acts in an alkaline medium, alkali albumin is first formed; but this is a very transient stage, the alkali albumin being quickly changed into deuteroalbumose.

[3] Otto, *Ztschr. f. physiol. Chem.*, Strassburg, 1883, Bd. viii. S. 129; Neumeister, *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 398.

[4] *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 381.

*hemi*peptone. It will be seen from this that the term hemipeptone is a term for something which has a separate existence only in theory. There has as yet been no method either devised or fallen upon by accident of separating these two substances which are supposed by the cleavage theory to be present, mixed in equal proportions, in amphopeptone. This is somewhat remarkable, in view of the number of years the theory has now been in vogue, and the large amount of experimental work that has been carried out in connection with it, and ought to be looked upon as an indication, either that amphopeptone is not really a mixture of antipeptone with a hypothetical hemipeptone, but a substance capable of breaking up under the action of trypsin into a new peptone (antipeptone) and a number of amido-compounds; or that anti- and hemipeptones are not separately present in amphopeptone, but that this peptone breaks up upon the further action of trypsin into antipeptone and hemipeptone, and that this hypothetical hemipeptone is next acted upon and broken into simpler bodies, finally yielding leucine, tyrosine, and the other companions of antipeptone found in complete tryptic digestion.

The decomposition of proteids by trypsin is represented by Neumeister[1] according to the following schema:—

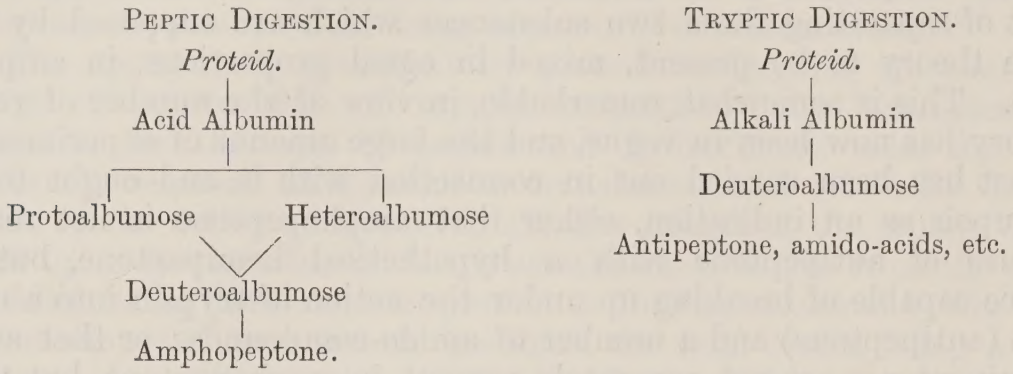


According to the same author, several deuteroalbumoses are formed, in the course of tryptic digestion, yielding corresponding amphopeptones. He also states that all the albumoses up to the present known, whether formed in peptic or tryptic digestion, are amphoalbumoses,—that is to say, yield both antipeptone and amido-acids on complete tryptic digestion. The ratio between the amounts of antipeptone and of amido-acids is a very variable one; heteroalbumose, for example, yielding much antipeptone and little amido-acid, while protoalbumose breaks up into much amido-acid and very little anti-peptone. Those who hold the cleavage theory explain this by saying that heteroalbumose is to a large extent an anti-substance, and protoalbumose almost purely a hemi-substance; but the experimental facts may be met equally well by the statement, that heteroalbumose is an albumose of such a chemical nature that it breaks up under the action of trypsin so as to yield a large percentage of peptone unalterable by further action of trypsin, accompanied by a small amount of amido-acids; protoalbumose is an albumose different in nature from heteroalbumose, and yielding, on further tryptic digestion, very little peptone (antipeptone) and a large amount of the amido-acids. There is no more proof that either heteroalbumose or protoalbumose is such a mixture of albumoses as the cleavage theory demands, than there is that amphopeptone is such a mixture of the corresponding peptones.

All the observed facts of peptic and tryptic digestion may be simply represented by the following schema, without any reference to the

[1] "Lehrbuch der physiologischen Chemie," Jena, 1893, Th. 1, S. 200.

cleavage or any other theory, save in the names of such of the substances as have been named on a theoretical basis :—



In the above account of the intermediate products formed between proteid and peptone, an attempt has been made to point out how far each important experimental result is in agreement with, or lends support to, the cleavage theory of proteid digestion. Most of the results have been obtained by supporters of that theory, but these results fall far short of proving the truth of the theory, and may be explained without reference to anti- and hemi-bodies. The main points may here be summarised :—

1. Certain substances have been obtained by the action of dilute acids on proteids, which do not yield amido-acids when subjected to prolonged tryptic digestion; these substances have been on this account looked upon as pure anti-compounds. But there is no evidence that such substances are formed naturally in either peptic or tryptic digestion : there is evidence against it in the extreme difficulty with which they are attacked either by pepsin or trypsin. Neither are these substances in their chemical behaviour albumoses, so that the term antialbumose, as applied to any of them, is a misnomer.

2. The substance originally obtained from a fractionated peptic digestion, and named hemialbumose, was afterwards shown by its discoverers to be a mixture of three bodies,—protoalbumose, heteroalbumose, and deuteroalbumose, —and none of these three discrete bodies was found to be either a pure hemialbumose or pure antialbumose, so that, if the cleavage theory is to be maintained, we must be content to believe that each of these three is a mixture in varying proportions of anti- and hemi-groups, and admit the existence of antiprotoalbumose and hemiprotoalbumose, of antiheteroalbumose and hemiheteroalbumose, of antideuteroalbumose and hemideuteroalbumose, without any experimental evidence whatever. Again, the cleavage theory takes no account of the fact that *proto-* and *hetero*albumose are formed prior to the deuteroalbumose.

3. Amphopeptone is supposed to be a mixture in about equal proportions of antipeptone and hemipeptone; but these two bodies have never been isolated from it. Antipeptone can only be obtained from amphopeptone by the action of trypsin, and hemipeptone has never been obtained at all.

4. There is no doubt that some forms of proteids, or altered proteids, are more easily decomposed by trypsin, yielding amido-acids, than are others; but this does not prove that such bodies are variable mixtures of a fraction which is not decomposable at all with one which is completely decomposable. When from an ampho-body there have been isolated two fractions, one a pure anti-body that is completely unalterable by trypsin, the other a pure hemi-body that is completely decomposable into amido-acids by trypsin, then it will be time to believe in ampho-, anti-, and hemi-bodies. At present neither from amphopeptone, protoalbumose, heteroalbumose, or deuteroalbumose has there

been such a separation, even partially, achieved, although these are admitted to be ampho-bodies by the supporters of the theory.

But if the cleavage theory be not accepted, what explanation is there for the fact that different albumoses yield varying accounts of amido-acids, which suffer varying amounts of decomposition, under the action of trypsin?

The different proteids, and the products derived from them, differ so little in chemical composition (and this is especially true for the various albumoses), that the difference in their nature is probably due to a difference in atomic grouping. Is it not probable, then, that some of these groups are much more susceptible of decomposition than others; that those albumoses which yield much amido-acid contain more groups in their molecules which are decomposable by trypsin; that those which yield much antipeptone contain less of these decomposable groups; and that in all cases that substance (or substances) which we call antipeptone is the remainder after all those groups which are attackable by trypsin have been removed in the form of amido-acids?

It will be seen that this substitutes, for two molecules, one easily attackable, the other wholly unattackable by trypsin, one molecule; of which a portion, variable in the case of each albumose, is attacked by the trypsin and a residue left, in which there are no groups that the trypsin is able to attack; such a substitution relieves one from belief in a large number of substances of which the existence has never been proven.

Again, if a cleavage of the proteid molecule takes place, at the beginning of the digestive process, into anti- and hemi-groups, of which the anti-groups, after passing through the albumose stage, become finally converted into antipeptone, while the hemi-groups, after passing through both albumose and peptone stage, become finally converted into amido-acids, one would expect, in an interrupted tryptic digestion, to find these intermediate hemi-products mixed with the intermediate anti-products; to find substances, corresponding to those found in peptic digestion, which would become on more complete tryptic digestion partially, at least, broken up into amido-acids. No such compounds or mixtures are, however, actually found; no hemi-compound is ever found at any stage of tryptic digestion. As already stated, *proto-* and *hetero-*albumose are never formed, only deuteroalbumose.

Neither is there any evidence of the formation of such a substance as amphopeptone in tryptic digestion, only antipeptone is formed. In short, there is no evidence whatever in tryptic digestion of two parallel series of anti- and hemi-bodies proceeding *pari passu* into anti- and hemi-peptones, of which the latter becomes decomposed into amido-acids. If any hemi-bodies are formed, they are at once broken down into amido-acids, without passing through the preliminary stages of hemialbumose and hemipeptone; at any rate, there is no experimental evidence of such a passage. Also, when protoalbumose is obtained as a product of fractional peptic digestion, and submitted to the action of trypsin, it is directly broken up into amido-acids, no deuteroalbumose or hemipeptone being discoverable as intermediate products. Similarly, heteroalbumose is in part converted into amido-acids, and in part into *anti*-deuteroalbumose, which passes later into antipeptone without any formation of *hemi*-deuteroalbumose or amphopeptone.[1]

[1] Neumeister, *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 381.

This is all easily accounted for on the supposition that a variable fraction of the proteid molecule is easily attacked and broken off into amido-acids by trypsin, but it is very difficult to explain on the supposition that the proteid molecule, early in the process of decomposition, breaks up into two halves, of which one changes through the stages of hemialbumose and hemipeptone into amido-acids, while the other, passing through antialbumose, halts at antipeptone.

Description of the products formed in the pancreatic digestion of proteids.—The products of tryptic digestion may be isolated most easily by experimenting with fibrin, either by impregnating it with the ferment, washing, and allowing it to digest in dilute sodium carbonate solution, or by digesting with a purified pancreatic extract. The products present at different stages may be studied by removing at intervals a portion of the digest, stopping the digestive process, by boiling and then investigating the nature of the dissolved substances.

Coagulable proteid.—If the test portion be removed before complete solution, or just on complete solution of the fibrin, it will be found to contain coagulable proteid; on neutralising, part of this, being a globulin in character, is thrown out of solution, and the remainder on making faintly acid and boiling.[1]

The deuteroalbumose of pancreatic digestion.—If, after removal of the coagulated proteid by filtration, the solution be now concentrated, deuteroalbumose can be precipitated from it by sodium chloride and acetic acid, and shown, by subjection to further action of trypsin, to be purely an anti-compound, or, in other words, to contain nothing in its molecule decomposable by the action of trypsin into amido-acids. This anti-deuteroalbumose, as already stated, is the only albumose found in tryptic digestion, and it is only found in the earlier stages. Another portion of the digest may be acidified, and the albumose thrown out of solution by saturation with ammonium sulphate, after which the presence of peptone in the filtrate may be shown, after dilution or dialysis, by the usual tests.

After some days of tryptic digestion, the digest contains no coagulable proteid or albumose, but only antipeptone, and the simpler products formed by more complete demolition of part of the proteid molecule (or of the hypothetical hemi-moiety), such as the amido-acids.

The peptone of tryptic digestion or antipeptone.—The peptone or peptones formed by the action of trypsin on proteids can best be obtained from a pancreatic digest which has been allowed to proceed to completion by repeated digestion during several days with trypsin and dilute sodium carbonate solution. This solution is concentrated to a small volume and filtered from the tyrosine, which separates out on cooling. The filtrate is saturated with ammonium sulphate, with the precautions described under peptic digestion,[2] and the ammonium sulphate is similarly removed. The antipeptone may now be precipitated by the addition of phosphomolybdic acid, the precipitate decomposed by baryta water, and excess of barium removed by cautious addition of dilute sulphuric acid. Finally, the solution is concentrated to a syrup on a water bath, and dried *in vacuo* over sulphuric acid.[3]

Antipeptone agrees very closely in composition and properties with a monobasic organic acid (Fleischsäure) recently isolated by Siegfried[4] from muscle extract, of the composition and molecular weight represented by the

[1] See pp. 405, 415.

[2] See p. 411.

[3] Kühne, *Ztschr. f. Biol.*, München, 1893, Bd. xxx. S. 1.

[4] *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, Math.-phys. Cl., 1893, S. 485; *Arch. f. Anat. u. Physiol.*, Leipzig, 1894, S. 401; *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 360. See also C. W. Rockwood, *Arch. f. Anat. u. Physiol.*, Leipzig, 1895, S. 1; Balke u. H. S. Ide, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xxi. S. 380.

formula $C_{10}H_{15}N_3O_5$. This substance gives a similar biuret reaction to that given by antipeptone; like it also, it does not give Millon's reaction, is very hygroscopic, and, on decomposition with hydrochloric acid, forms lysine and lysatinine, but not tyrosine. It has also been obtained directly from the products of advanced tryptic digestion; it has been found in milk, and in traces in the urine. It is easily soluble in water; sparingly in cold, more so in hot alcohol, from which it crystallises in microscopic crystals. It is also soluble in carbolic acid and glacial acetic acid, but is decomposed by these solvents, especially at a high temperature. It combines with hydrochloric acid and with phosphoric acid (Phosphorfleischsäure). The compound with phosphoric acid is the form in which it naturally occurs in the organism. Sjöqvist[1] has recently estimated the molecular weight of antipeptone by cryoscopic determination at 250; this agrees very closely with the molecular weight similarly determined by Siegfried for his new acid, and increases the probability that the two substances are identical.

When a proteid is subjected to tryptic digestion, a portion is decomposed beyond the stage of albumose or peptone, and there are formed several nitrogenous bodies of much simpler constitution; of these, some are amido-acids and some organic bases. Of these substances, two amido-acids, leucine or amido-caproic acid, and tyrosine or para-oxyphenylamido-propionic acid, are present in much larger quantity than the others, which only occur in traces. These others are aspartic acid or amido-succinic acid, glutamic acid or amido-pyrotartaric acid, butalanine or amido-valerianic acid; and of bases, ammonia, lysine, and lysatinine. Besides these substances of known composition, there is another substance of unknown composition formed, to which the name of tryptophan has been given, although it has never been isolated, and is only known through certain peculiar colour reactions which it gives.

The amido-acids formed in tryptic digestion.[2]—**Leucine.**—Leucine is an amidocaproic acid ($(CH_3)_2CH.CH_2.CH(NH_2).COOH$), and is always formed in any profound decomposition of proteid, such as boiling with dilute acids or alkalies, fusing with alkalies, in tryptic digestion, or in putrefaction. It has been found in nearly all the tissues in the body, and there has been much discussion as to whether it is a normal constituent here, or is formed as a post-mortem product. Certainly it is rapidly increased in amount, because of proteid decomposition, after death, but the evidence is strong for its normal presence in more or less pronounced traces in most of the organs in the fresh condition. It is, besides, a very common constituent of tissue in many pathological conditions, and also occurs in the vegetable world.

Virchow showed that both leucine and tyrosine are found normally in the pancreas after death, and Kühne afterwards showed that its amount here was much increased by auto-digestion of the gland tissue post-mortem.

Leucine was first discovered by Proust in 1818 in putrefying cheese, and named by him cheese oxide (Käse-oxyd). It was also obtained by Braconnet by decomposing animal matter with sulphuric acid.[3]

Leucine may be prepared in many ways: by tryptic digestion of proteids, by boiling various forms of proteid with dilute acids or alkalies, with stannous chloride and hydrochloric acid, with bromine water in sealed tubes, or by fusing with caustic alkalies. A common method is that of boiling horn shavings with dilute sulphuric acid for many hours; but any form of proteid will yield it when so treated, such as meat, cheese, fibrin, wool, feathers, elastic tissue.

Leucine has been obtained artificially by Limpricht,[4] by acting on isoval-

[1] *Skandin. Arch. f. Physiol.*, Leipzig, 1896, Bd. v. S. 277.

[2] For a very full account of these bodies, see Gamgee, "Physiological Chemistry of the Animal Body," vol. ii. p. 231.

[3] Maly, Hermann's "Handbuch," Bd. v. (2), S. 207.

[4] *Ann. de chim.*, Paris, 1854, tome xciv. p. 243.

eraldehyde with hydrocyanic and hydrochloric acids. Isovaleraldehyde (C_4H_9COH) is prepared, according to the general method, by oxidising amyl alcohol with potassium bichromate and sulphuric acid; purified by forming the sodium bisulphite compound, decomposing this and collecting the distillate; this is shaken with ammonia, when isovaleraldehyde-ammonia is thrown down in crystalline form. These crystals are washed with water, and then boiled with a mixture of strong hydrocyanic and dilute hydrochloric acids, when a reaction takes place yielding a body of the composition $C_{18}H_{33}N_5$, which breaks up into leucine and ammonia.

$$C_{18}H_{33}N_5 + 6H_2O = 3(\underset{\text{(leucine)}}{C_6H_{13}NO_2}) + 2\ NH_3.$$

Leucine has also been obtained artificially by Hüfner,[1] by heating monobromocaproic acid with saturated ammonia under pressure to 120°–130° C. during four or five hours.

$$C_5H_{10}BrCOOH + NH_3 = C_5H_{10}(NH_2)COOH + HBr.$$

Constitution of leucine.—That leucine is an amidocaproic acid is shown both by these methods of artificial preparation and by the following reactions:—

1. Heated under pressure to 140°–150° C., with strong hydriodic acid, it yields caproic acid, iodide of ammonium, and iodine.

$$\underset{\text{(leucine)}}{C_5H_{10}(NH_2)COOH} + 3\ HI = \underset{\text{(caproic acid)}}{C_5H_{11}COOH} + NH_4I + I_2$$

2. Heated alone, rapidly over its melting-point (170° C.), to 180°–200° C., it yields amylamine and carbon-dioxide.

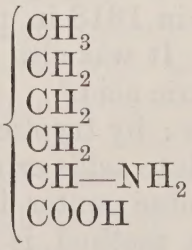
$$C_5H_{10}(NH_2)COOH = C_5H_{11}NH_2 + CO_2$$

3. When acted upon by nitrous acid, it breaks up in the usual manner of amido-acids, all the nitrogen being evolved as such, and oxycaproic or leucic acid being simultaneously formed.

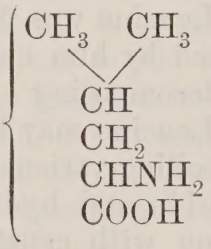
$$\underset{\text{(leucine)}}{C_5H_{10}(NH_2)COOH} + HNO_2 = \underset{\text{(leucic or oxycaproic acid)}}{C_5H_{10}(OH)COOH} + H_2O + N_2$$

These reactions show that leucine is an amidocaproic acid, but there are several isomeric amidocaproic acids.[2] It was thought until quite recently that leucine was the amido-acid of normal caproic acid, but it has been recently shown to be amido-isobutylacetic acid.[3] The difference in the structure of these two compounds would be represented according to the usual convention by the two following graphic formulæ:—

Normal α-amido-caproic acid



Isobutyl-(α) amidoacetic acid, or leucine



Pure leucine crystallises in the form of thin white transparent plates, forming in mass a snow-white powder, which feel greasy and are not wetted

[1] *Chem. Centr.-Bl.*, Leipzig, 1869, S. 159; *Journ. f. prakt. Chem.*, Leipzig, 1870, Bd. i. S. 6.

[2] According to R. Cohn, not one but several leucines are formed in pancreatic digestion; these are probably the isomeric amidocaproic-acids, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xx. S. 203.

[3] Schulze and Likiernik, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1891, Bd. xxiv. S. 669; B. Gmelin, Inaug. Diss., Tübingen, 1892.

by water, so that they float on its surface, although their specific gravity is about 1·3 ; but usually leucine is found to separate from solutions containing it in characteristic globules of microscopic size, often exhibiting a radial striation, or a marking off into concentric alternately dark and light bands. In this latter impure form it is easily soluble in water, and fairly so in alcohol ; the pure product is less soluble, its solubility is variously stated from 1 in 29 to 1 in 47 parts of water at room temperature. This difference is usually ascribed to the presence of different isomeric modifications in varying proportions.

Heated slowly to 170° C., leucine melts and commences to sublime in loose woolly flocks, resembling those formed when zinc is burnt to zinc oxide ; these present the appearance microscopically of thin plates grouped into rosettes. Leucine is very feebly soluble in strong alcohol (about 1 in 1000 in 98 per cent. alcohol), and is insoluble in ether.

The artificial leucine obtained as described above is inactive ; so is that obtained by the action of barium hydrate on proteids at high temperatures (150°–160° C.). Leucine from the tissues is dextrorotatory, but also becomes inactive when heated to 150° C. with baryta water. When Penicillium glaucum is sown in inactive leucine, the organism lives on the dextrorotatory variety, and laevorotatory leucine is left behind. These two are physical isomers of each other ; their specific rotatory powers are $(\alpha)\text{-D} = +17{\cdot}3$ for the right-handed, and $(\alpha)\text{-D} = -17{\cdot}5$ for the left-handed.[1]

Tests for leucine.—Leucine may be recognised—

1. By its crystalline form in the above-described spherules, forming from solution, and yielding a woolly sublimate which shows rosettes of platelets under the microscope. If it be heated rapidly so as to raise the temperature much above 170°, in subliming it the odour of amylamine is obtained.

2. By dissolving in boiling water and adding boiling solution of cupric acetate, when a deep blue coloured crystalline compound appears.

3. By Scherer's test, which consists in adding a drop of nitric acid and slowly evaporating on platinum foil, when a nearly colourless residue is left. If this be wetted with sodium hydrate and gently heated, it forms into an oily globule which rolls about on the foil.

Tyrosine.—Tyrosine, or *para*-oxyphenyl-*a*-amidopropionic acid ($C_6H_4(OH)CH_2CH(NH_2)COOH$), is the almost constant companion of leucine in the decomposition of proteids. Unlike leucine, tyrosine is never found as a constituent of *fresh* tissues ; its supposed presence in fresh pancreas has been shown to be due to self-digestion of the gland,[2] and it is not found in other fresh tissues, but is a constant constituent of those in which proteid decomposition has set in. It occurs very plentifully in old cheese, from which it was first obtained by Liebig by fusing with caustic potash.

Tyrosine may be obtained in general by the same methods as leucine, but it is not formed in the decomposition of gelatin nor of antipeptone.

Constitution of tyrosine.—The constitution of tyrosine has been established mainly by the work of von Barth,[3] who first showed that tyrosine yielded on fusing with caustic potash *para*-oxybenzoic acid, an isomer of salicylic acid. Previously to this, tyrosine had been looked upon as a derivative of salicylic acid, but from this von Barth concluded it must be ethylamido-*para*-oxybenzoic acid ($C_2H_5.NH.C_6H_3.OH.COOH$). If this formula were correct, on treating with hydriodic acid, ethylamine ($C_2H_5.NH_2$) ought to be obtained, but Hüfner showed that ammonia instead was split off. Von Barth next found that the

[1] Schulze and Boshard, *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 63 ; 1886, Bd. x. S. 134.

[2] Radziejewski, *Virchow's Archiv*, 1866, Bd. xxxvi. S. 1 ; Kühne, *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, Bd. i. S. 317.

[3] *Ann. d. Chem.*, Leipzig,, Bd. cli. S. 100. See also Erlenmeyer u. Lipp, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1882, Bd. xv. S. 1544.

NH_2 group in his reaction was not replaced by hydrogen but by hydroxyl, and so finally arrived at the formula $C_6H_4.OH.C_2H_3(NH_2)COOH$, which is in agreement with all the experimental facts, and is now universally accepted.

When fairly pure, tyrosine crystallises in long slender needles, which occur both singly and in double sheaves or in rosettes. If impure, however, it very often separates in balls or nodules closely resembling those of leucine, recrystallising from warm water in the crystalline form above described; if the solution containing the crystals be filtered, these felt themselves together on the surface of the paper to a thin, snow-white, paper-like mass. Tyrosine is much more insoluble in water than leucine (1 in 1900 of cold water), more so (1 in 150) in boiling water and in dilute and concentrated mineral acids, and also in alkaline solutions (ammonia, alkalies and their carbonates, and the alkaline earths). Tyrosine exhibits the usual facility of amido-acids for forming compounds, both with bases and acids; the copper compound is sparingly soluble in water, and is formed in dark blue needles on the addition of freshly precipitated cupric hydrate to a boiling solution of tyrosine, and allowing to cool.

Tyrosine, unlike leucine, cannot be sublimed without decomposition, and on dry distillation yields carbon-dioxide and a base of the composition $C_8H_{11}NO$.

Tests for tyrosine.—Tyrosine may be identified by the following tests:—

1. Its crystalline form.

2. *Scherer's test*, which consists in evaporating a portion with strong nitric acid in a platinum dish, leaving a transparent deep yellow residue, which turns red on moistening with caustic soda solution, and then a blackish brown on again evaporating.

3. *Piria's test.*—A drop or two of strong sulphuric acid is added to the tyrosine in a watch-glass; after half-an-hour, during which tyrosine sulphuric acid forms, the acid is diluted with water, and neutralised by the addition of calcium carbonate. The solution is filtered from the calcium sulphate so formed, and a drop of neutral ferric chloride solution added, when a deep violet colour appears, similar to that given by salicylic acid.

4. *R. Hoffmann's test.*—This is really identical with the Millon test for proteids, and in cases where there is no group present in the proteid molecule capable of yielding tyrosine, the test with Millon's reagent does not succeed, *e.g.*, in the case of gelatin and of antipeptone. The test may be carried out directly in the case of tyrosine itself, by boiling a solution containing this with Millon's reagent, when the solution passes through pink into deep crimson.

Separation of leucine and tyrosine.—Leucine and tyrosine may very easily be separated when in solution together by means of their very different solubilities. To separate them after pancreatic digestion, it is best to allow digestion to proceed for several days; at the end of this time there is no coagulable proteid, or albumose, except in traces, left in the solution. This is neutralised and evaporated down, when the tyrosine, on account of its sparing solubility in water, is thrown out in crystalline masses, while the more soluble leucine nearly all remains in solution; on cooling, more of the tyrosine separates out, and when the solution is cold it is filtered off, extracted with hot alcohol to remove traces of leucine, and purified by recrystallisation from hot water, or by dissolving in weak ammonia and precipitating by neutralisation.

The filtrate containing the leucine and peptone is still further evaporated until it becomes syrupy; it is then extracted with boiling alcohol, which takes up only the water and leucine. On evaporating off the alcohol, leucine is thrown out of the concentrated solution, and may be purified by sublimation or by repeated recrystallisation from alcohol. More tyrosine may be obtained from the residue left by the boiling alcohol.

Or the solution, after completion of digestion and careful neutralisation, may at once be evaporated to a thin syrup and set aside for twenty-four hours,

during which time most of both the leucine and tyrosine crystallises out. After separation of the crystals, the filtrate may be once more reduced in bulk by evaporation and a second crop of crystals obtained as before.

To the syrupy mother-liquor now remaining absolute alcohol is added, until precipitation of the peptone commences, when the addition of alcohol is stopped and the precipitate of peptone redissolved by gently warming. The solution is now set aside to cool and crystallise as before. The united crops of crystals of mixed leucine and tyrosine are boiled with alcohol, which dissolves the leucine and but little of the tyrosine. On concentrating this alcoholic extract, leucine crystallises out and may be purified by recrystallisation from alcohol. From the residue insoluble in alcohol the tyrosine is obtained by dissolving in weak ammonia water and neutralising.

The yield, both of leucine and tyrosine, obtained from different materials varies greatly, but in all cases the former is always formed in much larger quantity. The following table[1] gives the percentage yield of the substances obtained in some cases; the figures indicate parts per 100:—

Source.	Leucine.	Tyrosine.	Observers and Method.
Gelatine . . .	1·5–2 (*a*)	None (*a*)	(*a*) *Nencki*, boiling with dilute sulphuric acid.
Ligamentum nuchæ	36–45 (*b*)	0·25 (*b*)	(*b*) *Erlenmeyer* and *Schöffer*, boiling for some hours 1 pint of material, 2 pints sulphuric acid, 3 pints water.
Fibrin . . .	14·0 (*b*)	2·0 (*b*)–3·3 (*e*)	(*c*) *Hlasiwetz* and *Habermann*, heating with bromine under pressure.
Muscle . . .	18·0 (*b*)	1·0 (*b*)	(*d*) *Städeler*, heating with sulphuric acid.
Horn . . .	10·0 (*b*)	3·6 (*b*)–4·0 (*d*)	(*e*) *Schutzenberger*, heating with baryta water for four to six days, at 160°–200° C.
Egg albumin . .	22·6 (*c*)	1·0 (*b*)–2·0 (*e*)	
Plant albumin .	17·3 (*c*)	2·0 (*e*)	
Casein . . .	19·1 (*c*)	4·1 (*e*)	
Fibrin . . .	7·9 (*f*)	3·3 (*f*)	(*f*) *Kühne*, digestion of boiled fibrin.

Aspartic acid, or amido-succinic acid [$C_2H_3.(NH_2).(COOH)_2$], does not occur in any of the animal tissues or secretions, but is formed in small quantity in all those decompositions of proteids and their allies already described as furnishing leucine and tyrosine.[2] It was first identified among the products of pancreatic digestion of fibrin by Radziejewski and Salkowski,[3] and von Knieriem afterwards showed that it is also formed in the pancreatic digestion of plant glutin.

It may also be obtained by decomposing asparagin (amido-succinamic acid) by an alkali or acid, thus:—

$$\begin{array}{l} CH_2—COOH \\ | \\ CH(NH_2)—CO(NH_2) + HCl + H_2O \end{array} = \begin{array}{l} CH_2—COOH \\ | \\ CH(NH_2)—COOH \end{array} + NH_4Cl.$$

(asparagin or amido-succinamic acid). (aspartic acid or amido-succinic acid).

[1] Compiled from Maly, Hermann's "Handbuch," Bd. v. (2), S. 209 *et seq.*

[2] Ritthausen and Kreuster, *Journ. f. prakt. Chem.*, Leipzig, 1871, Bd. iii. S. 314; Hlasiwetz and Habermann, *Ann. d. Chem.*, Leipzig, 1871, Bd. clix. S. 304.

[3] Radziejewski and E. Salkowski, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1874, Bd. vii. S. 1050; *Ann. d. Chem.*, Leipzig, 1873, Bd. clxix. S. 150; W. v. Knieriem, *Ztschr. f. Biol.*, München, 1875, Bd. xi. S. 198. From 100 pts. of dry egg albumin Hlasiwetz and Habermann obtained 23·8 pts. of aspartic acid by the action of bromine in sealed tubes.

Aspartic acid is soluble with difficulty in cold water, easily soluble in boiling water, and insoluble in alcohol. It crystallises in rhombic prisms; its solutions are optically active, and curiously when in acid solution it is dextrorotatory, but laevorotatory when in alkaline solution. It forms a crystalline compound with copper, which may be used for purifying it. After leucine and tyrosine have crystallised out from the products of a proteid decomposition, they are separated from the mother-liquors, and these are further concentrated and treated with a small quantity of alcohol, when after a time a new crust of crystals forms. These are dissolved in water, the solution is boiled with freshly precipitated cupric hydrate and filtered; in the filtrate, on cooling, crystals are deposited of the copper salt of aspartic acid just mentioned. These crystals are dissolved in hydrochloric acid, the copper is thrown out by a stream of sulphuretted hydrogen, and the copper sulphide filtered off; in the filtrate, crystals of aspartic acid separate out.

Glutamic acid is amido-pyrotartaric acid [$C_3H_5.(NH_2).(COOH)_2$], and is homologous with aspartic acid, being the next higher member in the series. It occurs in minute quantities in the artificial decomposition of proteids, but has not yet been shown to be formed in the decomposition brought about by pancreatic digestion. It has been obtained by Ritthausen and Kreuster,[1] in the decomposition of vegetable proteid by dilute sulphuric acid; from casein when decomposed by stannous chloride and hydrochloric acid, by Hlasiwetz and Habermann;[2] and from reticulin, by Siegfried.[3]

It may be obtained by saturating its ice-cold solution with hydrochloric acid gas, and then keeping in a freezing mixture until the compound with hydrochloric acid ($C_5H_9NO_4 + HCl$) separates out in crystals, which are sparingly soluble in saturated hydrochloric acid, but easily soluble in water. Next, these crystals are dissolved in warm water, and the boiling solution is treated with freshly precipitated moist silver oxide, which removes the hydrochloric acid by forming silver chloride; the filtrate is freed of silver by a stream of sulphuretted hydrogen, and concentrated. On standing, glutamic acid separates in crystals which form rhombic tetrahedra or octahedra, sparingly soluble in cold, readily soluble in hot water, but insoluble in alcohol or ether. Solutions of the acid are dextrorotatory $(\alpha)D = +31{\cdot}1$, and it shows the same phenomena with regard to rotation as are described for leucine.[4]

Organic bases formed in tryptic digestion.—Lysine and **lysatine** or **lysatinine.**—Two organic bases, lysine and lysatine or lysatinine, have been recently isolated from the products of artificial decomposition of proteids, by means of a modification of the method of Hlasiwetz and Habermann, in which metallic zinc was added in addition to stannous chloride and hydrochloric acid, and means taken to exclude oxygen during the operation. These substances were first isolated from casein by Drechsel,[5] and afterwards extensively studied by himself and others.[6] They have since been found among the products of tryptic digestion.[7]

Lysine and lysatine are both precipitated by a hot saturated solution of phosphotungstic acid, which does not precipitate the amido-acids, and so furnishes a means of separating the two from the other products of a proteid

[1] *Journ. f. prakt. Chem.*, Leipzig, 1871, Bd. iii. S. 314.
[2] *Ann. d. Chem.*, Leipzig, 1873, Bd. clxix. S. 150.
[3] "Habilitationsschrift," Leipzig, 1892.
[4] See p. 423.
[5] *Arch. f. Physiol.*, Leipzig, 1891, S. 254; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1890, Bd. xxiii. S. 3096.
[6] E. Fischer, *Arch. f. Physiol.*, Leipzig, 1891, S. 265; Max Siegfried, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1891, Bd. xxiv. S. 418; *Arch. f. Physiol.*, 1891, S. 270; S. G. Hedin, *ibid.*, 1891, S. 273; Drechsel and Krüger, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1892, Bd. xxv. S. 2454.
[7] Hedin, *Arch. f. Anat. u. Physiol.*, Leipzig, 1891, S. 273.

decomposition. Lysine forms a platinochloride ($C_6H_{14}N_2O_2$, $H_2PtCl_6 + C_2H_5OH$) which is insoluble in 50 per cent. alcohol, in which the corresponding lysatine salt is soluble, and by this means the two bases may be separated; or they may be separated by means of the difference in solubility of their silver salts.[1]

Lysine, $C_6H_{14}N_2O_2$, in composition corresponds to a diamido-caproic acid ($C_5H_9(NH_2)_2COOH$); its solutions are dextrorotatory, but, like leucine and glutamic acid, become inactive when heated with baryta water to 150° C. The salts of lysine are crystalline, but the base itself has not been obtained in a crystalline form.

Lysatine or lysatinine yields a crystalline silver salt of the composition $C_6H_{13}N_3O_2$, $HNO_3 + AgNO_3$, from which the formula of the base follows as $C_6H_{13}N_3O_2$, except, as is supposed probable, the silver salt contains a molecule of water of crystallisation, in which case the formula of the base would be $C_6H_{11}N_3O$. With the former formula it would be homologous with creatine, with the latter homologous with creatinine, and would be most properly called lysatine or lysatinine accordingly.

Creatine is $C_4H_9N_3O_2$ and creatinine is $C_4H_7N_3O$. The new base may be either lysatine with the formula $C_6H_{13}N_3O_2$, or lysatinine with the formula $C_6H_{11}N_3O$; in either case being the second higher number in a homologous series, that is differing in formula by $(CH_2)_2$.

Another similarity to creatine invests this organic base with its most important physiological interest. Creatine when boiled with baryta water splits up into sarcosin (or methyl-glycocoll) and *urea*; similarly treated, lysatine also yields urea. Drechsel treated the lysatine obtained from 10 grms. of the silver salt above referred to with excess of baryta water, and obtained 1 grm. of urea nitrate, from which he isolated and identified the urea. This is all the more interesting from the fact that creatine, although it occurs in the body under such circumstances as leave little doubt that it is formed as a decomposition product of proteids, has not yet been obtained artificially as a direct product of proteid decomposition. Lysatine has not only been so obtained, but also as a product of pancreatic digestion, and urea having been obtained from this, has consequently been obtained as a product of proteid decomposition.

Hedin[2] obtained from 3 kilos. of moist fibrin, 28 grms. of pure platinochloride of lysine, and enough of the silver salt of lysatinine to establish its identity.

Ammonia is found as a constant product in the artificial decomposition of proteids, as might be inferred from what has been stated concerning lysatine, and its formation has also been shown in pancreatic digestion. Hirschler[3] has shown that in the entire absence of putrefaction, in so short a period as four hours, small quantities of ammonia appear in the pancreatic digestion of fibrin; this result has been confirmed by Stadelmann.[4]

The chromogen of pancreatic digestion.—As early as 1831 it was observed, by Tiedemann and Gmelin,[5] that the pancreatic juice of the dog takes on a rose-red colour when mixed with chlorine water. Claude Bernard next showed that no such reaction is obtained with fresh pancreatic juice, but first appears after the juice has been kept for some time without putrefaction setting in; if putrefaction takes place, the reaction is also not obtained. The product giving this colour reaction is now definitely recognised as a product of pancreatic digestion, and not a constituent of pancreatic juice. For it the name trypto-

[1] For details of these processes see Gamgee, "Physiological Chemistry," London, 1893, vol. ii. p. 255.

[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1891, S. 273.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. x. S. 302.

[4] *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 261.

[5] "Die Verdauung nach Versuchen," Heidelberg, 1831.

phan has been suggested by Neumeister,[1] from the point of view that it may be made to serve as an indicator of when tryptic digestion has reached a certain stage and amido-acids are beginning to be formed,[2] since it first appears in the more advanced stages of proteid decomposition simultaneously with the amido-acids. Tryptophan has never been isolated, and is only known by its colour reactions. When not very dilute, the rose-red colour is replaced by violet, and Kühne has shown that the colour is given by bromine water as well as by chlorine water. According to Krukenberg,[3] the colour is not due to oxidation by the chlorine or bromine, but to the formation of an addition compound; he also states that tryptophan is slightly soluble in alcohol, ether, and chloroform. Hemala[4] has shown that the coloured material is easily soluble in amyl alcohol. Here chlorine and not bromine water must be used as a test, for the latter itself imparts colour to amyl alcohol. When much peptone or other impurity is present with it in solution, it falls, after some time, as a precipitate; this on shaking up with alcohol gives a fine violet solution showing an absorption band at the D line. According to Krukenberg, a strong coloration is given even by traces of the chromogen; he also has shown that tryptophan is diffusible.

In its reaction with bromine and chlorine water, tryptophan closely resembles the chromogen of the suprarenal gland; the two chromogens are also alike in being diffusible and in their powerful tinctorial action, but here resemblance ceases. The chromogen of suprarenals is very easily destroyed by alkalies, could not be formed in pancreatic digestion, and is quite insoluble in dry alcohol, ether, or chloroform.

Kühne has shown that tryptophan is a constant product in all proteid decomposition, but that it is rapidly destroyed and disappears; it is also rapidly destroyed by putrefactive changes.

When pancreatic digestion is accompanied by putrefaction, many other substances are formed besides those above described. These will be considered in connection with bacterial digestion in the intestine.

Digestion of Various Bodies allied to the Proteids.

Those substances, such as the mucins and nucleo-proteids, which consist of a proteid molecule united to some organic radicle (and called *Proteïde* by Hoppe-Seyler), first undergo a cleavage into proteid and the body involved with it; the proteid is then digested in the usual fashion, while the other substance very often suffers no change. In this manner hæmoglobin is decomposed by peptic digestion into a proteid commonly supposed to be a globulin, which becomes converted through albumose into peptone, and hæmatin which remains unchanged. Nucleo-proteids and nucleo-albumins[5] yield on similar treatment an insoluble residue of nuclein, or of pseudo-nuclein or paranuclein respectively, and the proteid part of the molecule is peptonised. In the tryptic digestion of fibrin some of the xanthin bases (or nuclein bases) have been found; these arise from the breaking up of nuclear-nuclein (*Kernnuclein*) present as a constituent of admixed nucleo-proteid, derived from the nuclei of white blood corpuscles. The nuclein breaks up into nucleic

[1] *Ztschr. f. Biol.*, München, 1890, Bd. xxvii. S. 309.

[2] The name proteinochromogen has been given to this chromogen by Stadelmann, *ibid.*, 1890, Bd. xxvi. S. 491.

[3] Krukenberg, *Virchow's Archiv*, 1885, Bd. ci. S. 555; *Verhandl. d. phys.-med. Gesellsch. zu Würzburg*, 1884, S. 179.

[4] *Loc. cit.* See also Neumeister, *Ztschr. f. Biol.*, München, 1890, Bd. xxvi. S. 332.

[5] Nucleo-proteids yield on decomposition a true nuclein, containing nucleic bases, nucleo-albumins a pseudo-nuclein or paranuclein, which does not contain such bases.

acids and proteid, and the nucleic acids in their turn into nuclein bases and phosphoric acid. These changes take place very slowly in tryptic digestion. On digestion with pepsin and hydrochloric acid, the glycoproteids are decomposed, yielding a carbohydrate substance which reduces Fehling's solution and a proteid which as before is peptonised. This decomposition only takes place slowly, and is probably due in great part to the feeble hydrolytic action of the hydrochloric acid.

The caseinogen of milk is first coagulated by the action of the rennin of the gastric juice, and afterwards the insoluble casein formed in this process is digested.

Casein is broken up in the process of gastric digestion into a proteid and pseudo-nuclein, of which the former is changed into peptone, while the latter is thrown out as an insoluble precipitate.

This precipitate corresponds to the dyspeptone of Meissner, and has been the subject of a considerable amount of investigation. Lubavin [1] found that it contained inorganic phosphorus, and that it is a mixture of which one part is soluble in dilute sodic carbonate (Na_2CO_3), while the other is insoluble. The soluble part contains 4·6 per cent. of phosphorus, and is probably identical with Hoppe-Seyler's nuclein. Chittenden [2] and others state that dyspeptone does not contain much phosphorus, and that this is probably present as calcium phosphate, dyspeptone being therefore not a nuclein but a mixture of calcium phosphate with a hydration product of casein. C. Wildenow [3] does not hold with this view, having obtained dyspeptone which contained only 0·13 per cent. of calcium, and 3·85–4·66 per cent. of phosphorus, but agrees with Lubavin that the precipitate is a nuclein. E. Salkowski [4] supports this conclusion; he also announces that on prolonged digestion the precipitate redissolves to a clear solution, part of the phosphorus being split off as phosphoric acid, and part remaining in organic combination (probably as paranucleic acid). Such a solution can be brought about, according to Salkowski, by a strong peptic solution within forty-eight hours.

The albuminoids as a class are fairly resistant to the action of digestive agents; when they are broken up, they yield products closely resembling those furnished by the decomposition of the true proteids.

Collagen is said to be converted into its hydrate gelatin more rapidly by the action of pepsin and hydrochloric acid than it would be by the acid alone; the gelatin thus formed is then acted upon by the pepsin and hydrochloric acid, and rapidly loses its characteristic property of gelatinising on cooling.[5] This physical change is the visible sign of a chemical one, by which the gelatin is converted into a substance called protogelatose; this is again changed, yielding deuterogelatose; and finally gelatin peptone is formed.[6] These substances resemble the corresponding compounds of proteid digestion, the gelatin peptone being distinguished from the other two products by its indifference to the saturation of its solutions with neutral salts and by its diffusibility. Protogelatose is thrown out of solution by saturation of its acidified solution with sodium

[1] *Med.-chem. Untersuch.*, Berlin, 1871, S. 463.
[2] *Stud. Lab. Physiol. Chem.*, New Haven, 1890, vol. iii. p. 66.
[3] Inaug. Diss., Bern, 1893.
[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1893, Nos. 23, 28; *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 401.
[5] J. de Bary, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, S. 75; Etzinger, *Ztschr. f. Biol.*, München, Bd. x. S. 84; Uffelmann, *Deutsches Arch. f. klin. Med.*, Leipzig, Bd. xx. S. 535.
[6] Chittenden and Solley, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 23.

chloride, while deuterogelatose is only precipitated by saturation with ammonium sulphate. Protogelatose is also precipitated by platinic chloride, while deuterogelatose is not so precipitated.

Collagen is not attacked by pancreatic juice unless it has been previously boiled with water, or swollen by the action of dilute acids, as it normally would be by the gastric juice.[1] This result is confirmed by the observation of Ludwig and Ogata, that after removal of the stomach proteid was still digested, but connective tissue was not attacked. After such preliminary treatment collagen is easily converted into gelatin, and the after course of events closely resembles that described for peptic digestion. There is first formed protogelatose, then deutero-gelatose, and finally gelatin peptone, which is not converted by any further action of trypsin into amido-acids.[2] Trypsin acts so easily on gelatin, and deprives it so readily of its power of gelatinising, that this has been recommended by Fermi as a test for trypsin.[3]

The decomposition products of gelatin have been long known, though not with the exactitude above described. Gmelin showed that it was decomposed by superheated steam at 140° C., and Hofmeister [4] obtained, after boiling with water in 1 per cent. solution for thirty hours, two cleavage products which he termed semiglutin and semicollin; these are probably identical with proto- and deuterogelatose.

Elastin is also dissolved by pepsin and hydrochloric acid,[5] though with more difficulty than collagen. The products of the peptic digestion of elastin were studied by Horbaczewski,[6] who described two products which he called hemielastin and elastin peptone. The same subject has been investigated more recently by Chittenden and Hart,[7] who have shown that two substances are formed in the peptic digestion of elastin, but that both these substances are albumoses, since they are both precipitated by saturation of their solutions with ammonium sulphate; to these substances they gave the names of protoelastose and deutero-elastose. The former is precipitated on saturation of its solution with sodium chloride, while the latter is only precipitated on the addition of acetic acid. Elastin is also directly attacked by trypsin and dissolved, forming in turn proto- and deuteroelastoses as in peptic digestion, but neither in peptic or tryptic digestion is there any peptone formed.[8]

[1] Ewald and Kühne, *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1877, N. F., Bd. i. S. 451.
[2] Chittenden and Solley, *loc. cit.*
[3] *Arch. f. Hyg.*, München u. Leipzig, 1891, Bd. xii.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 299.
[5] Etzinger, *Ztschr. f. Biol.*, München, Bd. x. S. 84.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 330.
[7] *Ztschr. f. Biol.*, München, 1889, Bd. xv. S. 368.
[8] Chittenden and Hart, *loc. cit.*

THE ABSORPTION OF CARBOHYDRATES AND PROTEIDS.

It was for many years believed that the absorption of the products of digestion from the alimentary canal was governed by exactly the same physical laws as determine the passage of a solution and its dissolved constituents through an inert membrane, but the accumulation of experimental evidence has rendered such a belief no longer tenable. It is now known that the cells which line the alimentary canal take an active part, not only in absorbing the materials prepared for them by the action of the digestive secretions, but in modifying these products in various ways during the process.

Before the laws of diffusion of solutions were known, the process of absorption by the columnar cells of the intestine was compared by Tiedemann and Gmelin (1820)[1] to that of gland secretion. After the establishment of the laws of diffusion, attempts were made to apply them in explanation of absorption, as well as of other similar processes in the body. Such physical views persisted for a long time, until it was shown by conclusive experiments that absorption, like these other processes, does not obey the laws of physical diffusion, but is selective in its character and governed in some subtle way by the activity of the cells involved. Our modern view is thus, as is often the case, a recurrence to an older theory; the only difference being that we have a somewhat broader experimental basis on which to build it.

The cells of a secreting gland take up certain materials from the lymph in which they are bathed, and from these, in some manner, elaborate certain products which are passed into the gland lumen as a secretion. Similarly, the absorbing cells of the intestine take up certain products of digestion from the intestinal contents by which they are bathed, and build up from these certain materials which pass into the lymph. So that absorption may be regarded as a kind of reversed secretion.

In both cases the process is a selective one, the constituents of the gland secretion are definite in their nature, in many cases specific, and are probably formed from definite constituents of the lymph taken up by the secreting cell to the exclusion of others. In like fashion, certain materials only are taken up by the epithelial absorbing cell, and from these definite products are formed to be passed into the lymph.

That absorption is a selective process and not one of purely physical diffusion, is shown by the following observations:—

1. Certain colloids (*e.g.* alkali albumin) disappear from the intestine at a fairly rapid rate, even in the complete absence of digestive enzymes.[2]

2. The rate of absorption from the intestine of various dissolved substances is not proportional to their diffusion-coefficients. Sodium sulphate is much more diffusible than grape-sugar, but when a solution containing 0·5 per cent. of each of these is injected into the intestine, the sugar disappears much more rapidly, and only traces of it remain at a time when the greater part of the sodium sulphate is still left behind.[3]

3. The rapidity of absorption is much greater than can be accounted

[1] Quoted by Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Supp. Heft, Bd. xliii. S. 69.

[2] See form of absorption of proteids, p. 436.

[3] Röhmann, *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. xli. S. 411.

for, on the basis of physical diffusion from the intestinal contents to the lymph.[1]

4. If the dissolved products of digestion are carried through by diffusion, it must be passively in a diffusion stream due to salt diffusion, their own diffusive powers being too feeble to suppose they are carried by these. Now, not only would such a stream be too slow, but, in such a case, the amount of fluid which must be absorbed by the epithelial cells would be enormous. There is at the height of proteid digestion, even in an animal with such digestive powers as the pig, rarely more than 2 per cent. of albumoses and peptones together in solution in the intestine, and usually much less. If it be supposed that this is passively and not selectively absorbed, then to carry 100 grms. of digested proteid out of the intestine, 5 litres of water at least, and probably a great deal more, would be required. During the digestion of starch, only traces of sugar are found at any given time in the intestine, and generally it may be stated that absorption takes place from very dilute solution. There is no reason to believe that such enormous quantities of fluid are thrown into the intestine during digestion, to be afterwards absorbed from it, and hence it must be concluded that dissolved substances are not passively absorbed by their solutions passing unchanged into the epithelial cell.

Seat of absorption.—Absorption of some substances begins in the stomach,[2] but the main part takes place in the intestine. Water is practically not absorbed at all in the stomach,[3] while alcohol is readily taken up. The absorption of chloral hydrate and of sugar by the stomach is increased by the presence of alcohol.

Gastric absorption is said to be increased by greater concentration of the substance to be absorbed, while the reverse holds for intestinal absorption. A solution of grape-sugar is most rapidly absorbed from the intestine when its concentration lies at 0·5 per cent.; as the concentration increases from this the rate of absorption diminishes; while the rate of absorption in the stomach increases up to a concentration of 20 per cent.[4] According to v. Mering, all forms of sugar are absorbed in the stomach to a greater or less extent. The products of proteid digestion are also probably absorbed to a slight extent in the stomach.[5]

Channels of absorption.—The new materials formed by the action of the intestinal epithelial cells on the absorbed products of digestion, pass out of these cells into the lymphoid tissue of the villus underlying them. The modified carbohydrates and proteids pass in solution into the lymph which bathes the tissue, and in soluble form are absorbed from this lymph by the capillary vessels of the villus, thus passing directly into the portal circulation, while the fats leave the epithelial cells as fat globules, and are carried as such past the capillary network of the villus, to enter the lacteal situated in the axis of the villus.[6]

[1] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Supp. Heft, Bd. xliii. S. 70.

[2] See Busch, *Virchow's Archiv*, 1858, Bd. xiv. S. 171; Tappeiner, *Ztschr. f. Biol.*, München, 1880, Bd. xvi. S. 497; v. Anrep, *Arch. f. Anat. u. Physiol.*, Leipzig, 1881, S. 504; Meade-Smith, *ibid.*, Leipzig, 1884, S. 481; v. Mering, *Verhandl. d. Cong. f. innere Med.*, Wiesbaden, 1893; *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, Bd. viii. S. 533.

[3] Edkins, *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 445; v. Mering, *loc. cit.*; Gley and Rondeau, *Compt. rend. Soc. de. biol.*, Paris, 1893, p. 516.

[4] Brandl, *Ztschr. f. Biol.*, München, 1892, Bd. xxix. S. 277.

[5] See F. Hofmeister, *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 69.

[6] See "Digestion and Absorption of Fats," p. 457.

There are thus two channels of absorption leading to the systemic blood stream. One by the capillaries of the villus, passing through the liver; the other by the lacteals, *viâ* the abdominal lymphatics, to the thoracic duct leading directly to the subclavian vein.

Absorption of water.—It has been shown by Heidenhain[1] that by far the greater share of the water absorbed from the small intestine is taken up by the capillaries of the villus and not by the lacteals. When large quantities of dilute saline solution (0·3 per cent.) are injected into the small intestine, the rate of lymph flow in the thoracic duct is not markedly increased, unless so much salt solution is injected at one time that the intestine becomes forcibly distended. Zawilsky[2] also found that even during active fat absorption there was no great increase in the amount of lymph flowing from the thoracic duct; the lymph became charged with an exceedingly fine emulsion of fat, but was not largely increased in quantity.

The considerable absorption of water which commences in the lower end of the ileum, and goes on throughout the entire length of the large intestine, causing the thin chyme of the upper part of the small intestine to become semi-solid, and finally to assume the consistency of the fæces, is also carried out by the agency of capillary blood vessels, so that practically all the water absorbed from the intestine is taken up by the blood stream. The blood is not diluted to a corresponding extent in the process; in fact, even with the absorption of an excessive amount of water, as in Heidenhain's experiments above quoted, the composition of the blood is little altered. The absorbed water in such a case of excessive absorption passes at first into the lymph which bathes the tissues, to be afterwards brought out and gradually eliminated by the kidneys as the excess in the blood diminishes.

Absorption of soluble constituents.[3]—All those substances which leave the epithelial cell in solution, are also carried away from the lymph spaces of the villus by the capillaries.[4] This has been shown chiefly by observations made during active absorption of these several constituents, on the rate of flow in the thoracic duct, the constitution of the lymph so flowing, and the effects of ligature of the duct or diversion of the stream to the exterior. Direct analyses of the blood of the portal vein, as compared with the systemic blood, do not yield trustworthy results; partly because of the difficulty of making very exact determinations in such a complex fluid as blood serum; still more because of the very small change in composition which is sufficient to account for the carriage of a great weight of absorbed substance, by reason of the copious flow which takes place through the capillaries, especially when active digestion is in progress.

If a cannula be inserted into the upper end of the thoracic duct, and the rate of flow of the lymph stream measured, as well as the amount of proteid contained therein, neither of these is found materially to alter, whether the animal (dog) be fasting, or active proteid digestion be going

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Supp. Heft, Bd. xliii. S. 53; 1894, Bd. lvi. S. 579.

[2] *Arb. a. d. physiol. Anst. zu Leipzig*, 1876, Bd. xi. S. 161.

[3] For the Absorption of fats and fatty acids, see p. 443.

[4] It is often stated that all the dissolved intestinal contents are so absorbed, but if, as is probable, fats are absorbed in soluble form, such a statement is obviously incorrect. Only those constituents which remain soluble, *after* the action of the absorbing cells, pass into the capillaries.

on. This could obviously not be the case if any appreciable part of the proteid were absorbed by the lacteals.

Again, if the thoracic duct be ligatured, and an hour after the operation the animal (dog) be given a rich meal of proteid food, absorption goes on in a normal manner. If the animal be killed after the lapse of about forty-eight hours, it will be found that all the proteid has been absorbed, while a corresponding amount of nitrogen has been eliminated in the urine.[1]

A similar proof has been given for carbohydrate absorption by the blood vessels. In this case the animal is fed with carbohydrate food instead of proteid; and the amount of sugar in the lymph which flows from a fistula of the thoracic duct is estimated. The percentage of sugar lies between 0·6 and 1·6 per thousand, and does not vary in the least with the state of digestion, this being the usual percentage of sugar found in lymph or blood serum.[2]

A direct proof has also been given of the absorption of sugar by the capillaries, as it has been shown that on injection of sugar into the intestine the percentage of sugar in the portal vein may rise as high as 4 per 1000, while in a fasting condition the amount of sugar contained in the blood of either portal or hepatic veins does not essentially differ from that in the blood of the remainder of the circulation.[2]

It may be taken, then, that, under normal conditions, all the soluble constituents which leave the epithelial cell are taken up by the capillaries. But when excessive absorption is taking place, as when large quantities of sugar in concentrated solution are injected into the intestine, this is not the case. Here the work of absorption becomes too great for the capillaries, a part of the dissolved foodstuff passes the region of their action and is absorbed by the lacteals, probably in a passive fashion.

Conditions of absorption of carbohydrates.—There is no doubt that a considerable share of the carbohydrate food is taken up from the intestine by the absorbing cells as simple sugars (mainly as dextrose and lævulose), otherwise the reason of the ferment actions which have been previously described would be difficult to see. But we possess no experimental evidence to show that all the carbohydrate is absorbed in such a form. Indeed, it is probable that the absorbing cells are capable of taking up not only saccharoses, but even colloidal carbohydrates, such as dextrin and starch, and converting these into simple sugars before turning them into the blood stream.

We have already seen, in discussing the digestion of starch and glycogen, that it is impossible, in experiments carried out *in vitro*, to further convert all the dextrin formed into maltose or other form of sugar. Sheridan Lea's[3] experiments show, indeed, that the rapidity of diastatic action is much increased by dialysis, and the quantity of dextrin left unchanged into maltose largely diminished. Lea argues from this result that, under the more favourable conditions for removal of digestion products existing in natural digestion, the conversion of dextrin into maltose may become complete. Contrary to this view, there is the experience of Musculus and Gruber,[4] that the unchanged dextrin remaining after a prolonged digestion of starch, with a diastatic ferment, is not

[1] Schmidt-Mülheim, *Arch. f. Anat. u. Physiol.*, Leipzig, 1877, S. 549.
[2] Von Mering, *ibid.*, 1877, S. 379.
[3] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 226 ; see also pp. 321 and 394.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 177.

convertible into maltose by a fresh addition of diastatic ferment after complete removal of the maltose produced by the first digestion. So that the failure of the ferment to convert the last portion of dextrin into maltose cannot be wholly due to the stoppage of its action by the presence of excess of maltose.

There is no very evident reason why soluble materials like the dextrins should not be absorbed as such by the epithelial cells. The argument that dextrin is not directly assimilable, because, when injected subcutaneously or intravenously, it is eliminated by the kidneys, is not valid against its absorption as dextrin *by the epithelial cell.* For there is no reason to suppose that the cell must turn it into the lymph in exactly the same form in which it takes it up from the intestine; the chances are, in fact, all against such a supposition. It may be taken as probable, then, that the digestive enzymes of the alimentary canal are incapable of converting all the starch of the food into maltose, and hence into dextrose, and that a portion is absorbed as dextrin, and changed into something else before reaching the blood stream.

What has been said above concerning dextrin applies also to the double sugars. The intestinal juice, as we have seen, contains enzymes capable of converting maltose and cane-sugar into simple sugars, and it is probable that such a change does take place to a very large extent. Still it cannot be concluded that the double sugars undergo *complete* conversion before absorption. Lactose appears not to be acted upon by any of the digestive enzymes, and so far as it escapes lactic acid fermentation this double sugar must be absorbed by the epithelial cell unchanged. Again, Brown and Heron [1] found that the dried mucous membrane acted much more energetically on maltose than did any extract of it, which tends to show that this action takes place in part within the cell.

Rohmann [2] has also shown that not only sugar, but even starch solution disappears from a Thiry-Vella fistula with considerable rapidity; and as the succus entericus possesses only an exceedingly feeble diastatic action on starch, it seems that here the starch must be directly taken up by the intestinal cell. Such a view is also supported by the fact that, after removal of the pancreas, the secretion of which must produce the greater part of the diastatic action which goes on within the intestine, one-half to three-fourths of the starch of the food is still utilised.[3] Under normal conditions, however, the diastatic conversion by the pancreatic juice is so rapid, that it is very improbable that any appreciable portion of starch is absorbed as such.

Form in which carbohydrates reach the blood stream.—During active carbohydrate absorption, traces of carbohydrates, resembling dextrin, are said to be present in the blood of the portal vein,[4] but it is probable that very little carbohydrate leaves the epithelial cells other than dextrose or lævulose. These two sugars are capable of direct assimilation after subcutaneous injection, and of forming glycogen in the liver, but no such direct assimilation takes place in the case of cane-sugar or maltose.

[1] *Loc. cit.* [2] *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. xli. S. 411.

[3] Minkowski and Abelmann (Inaug. Diss., Dorpat, 1890; *Centralbl. f. Physiol.*, Leipzig u. Wien, 1891, Bd. iv. S. 522) found, after complete extirpation of the pancreas, an absorption of 57–71 per cent. of starch; the brothers Cavazzanni (*Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, Bd. vii. S. 217), under like circumstances an absorption of 47 per cent.

[4] Otto, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1888, Bd. xvii. S. 138; v. Mering, *Arch. f. Anat. u. Physiol.*, Leipzig, 1877, S. 413.

Ingestion of large quantities of solution of sugar leads to the appearance of sugar in the urine (alimentary glycosuria), due to the assimilation of the sugar not keeping pace with its absorption.[1] Absorption itself is also disturbed by the appearance of diarrhœa. But when carbohydrate is introduced into the alimentary canal, in the form of starch, immense quantities can be rapidly and completely absorbed without any glycosuria or other disturbance ensuing.

Thus Rübner[2] found that a man consuming a daily ration of 508–670 grms. of carbohydrate contained in wheaten bread, left unabsorbed only 0·8–2·6 per cent.; of the carbohydrate of peas (357–588 grms.) 3·6–7·0 per cent. was unabsorbed; and of potatoes (718 grms.) 7·6 per cent. This complete absorption and utilisation of carbohydrate, when taken in the form of starch, is probably due to the rate of assimilation and storage as glycogen in the liver, being able to keep pace with that of absorption from the intestine.

Conditions of absorption of proteids.—The power possessed by the intestinal cells of absorbing various forms of proteid affords one of the best illustrations that this process is not one of mere physical diffusion. The products found towards the end of a proteid digestion *in vitro* are distinguished from the proteids from which they originate by being slightly diffusible. To this fact great importance was at one time attributed, because it was thought that only proteids in a diffusible form were capable of absorption, and hence that peptonisation was in all cases a necessary preliminary. It is now generally admitted that many forms of native proteid are capable of entering the epithelial cells without previous change by digestion or otherwise; and in those cases in which a proteid is incapable of direct absorption a much less profound change than peptonisation is sufficient to render it so, namely, conversion into acid or alkali albumin. Such an absorption of soluble proteid, other than albumose or peptone, takes place not only in the small intestine, but in the large intestine, and even in the rectum.

Voit and Bauer[3] cleared a loop of small intestine of its contents as completely as possible by stroking, and separated it from the rest of the gut by double ligatures at each end. Various forms of proteid, in solutions of known amount and strength, were then injected into this loop; the intestine was replaced, and its contents examined on killing the animal (cat or dog) some hours later. It was found that variable amounts of these proteids had disappeared; thus, in one to four hours, 16–33 per cent. of white of egg had disappeared, and of syntonin from ox muscle 28–95 per cent. It might be supposed that the portion of proteid absorbed had been peptonised by traces of proteolytic enzyme which might be present in the intestine; but in the unabsorbed proteid remaining at the end of the experiment, no albumose or peptone was found. Voit and Bauer also injected solutions of white of egg and sodium chloride into the rectum of man and animals in a fasting condition, and found a marked increase (6 to 8 grms. in 24 hours) in the amount of nitrogen eliminated by the kidneys; in fact, an equilibrium of nitrogenous metabolism may even be maintained in this way. It has been shown by Eichhorst,[4] who confirms these results, that no appreciable amount of peptonisation takes

[1] C. Voit, *Ztschr. f. Biol.*, München, 1891, Bd. xxviii. S. 245.
[2] *Ibid.*, Bd. xix. S. 45.
[3] *Ibid.*, Bd. v. S. 562.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 570.

place in the large intestine. Finally, the objection that the action is due to traces of enzymes, has been disposed of by the observations of Czerny and Latschenberger,[1] in a case of a fistula situated in the sigmoid flexure. The rectum was thoroughly washed out from the fistula, yet from 60–70 per cent. of the injected proteid disappeared in 23–29 hours.

Assimilable and non-assimilable proteids.—Some forms of proteid, such as alkali albumin, prepared from white of egg, and acid albumin, prepared from muscle, myosin, fibrin, or white of egg, are directly assimilable; that is to say, when injected into the blood stream they are not removed again by the kidneys; others, such as unchanged white of egg, caseinogen, and glutin, are, when injected, at once excreted in the urine. The latter forms must therefore, under normal conditions, be changed during absorption, before passing into the blood; but when excess of white of egg is present in the intestine, absorption oversteps the rate at which this change can take place, and a portion of the egg albumin reaches the circulation unchanged. Under these circumstances, this portion is promptly removed by the kidneys, and an "alimentary albuminuria" is the result, just as an excessive amount of sugar in the intestine produces "alimentary glycosuria."

Relative amounts of proteids absorbed in different forms.—It is evident, then, that absorption can take place, either in the form of albumose or peptone, of alkali or acid albumin, or even occasionally in that of native proteid; and the question arises, to what extent does absorption take place under natural conditions in each of these different forms? Such a question is exceedingly difficult to answer by experiment. It is impossible to do so exactly by observation of the amount of each form of proteid present in the intestinal contents during proteid absorption, because the absorption is selective, and a substance present only in traces may be passing out of the intestine more rapidly as it is continuously formed than another which is present in much larger quantity.

A *rough* estimate of the relative amounts of proteid absorbed as albumose and peptone, and that absorbed in other forms, may be obtained from analyses of the intestinal contents during proteid digestion. Thus, Schmidt-Mülheim[2] examined the contents of the stomach and intestine at varying periods during digestion of flesh in dogs; he found that the amount of proteid in solution, both in the stomach and intestine, was small at any given time, *but that the amount present as albumose and peptone was always somewhat greater than that present in other forms.* When it is remembered that albumose and peptone are absorbed more rapidly than other proteids, this points to the greater part of the proteid being absorbed as albumose and peptone.

It is not known with certainty to what extent amido-acids are formed from proteids, in the natural course of intestinal digestion. The experimental evidence on the subject is somewhat conflicting, but the majority of observers are of the opinion that but little proteid is absorbed as leucine and tyrosine, being nearly all absorbed as albumose or peptone, or even at a still earlier stage.

The only positive evidence as to the formation of leucine and tyrosine in natural digestion, rests on the amounts found in the intestinal contents

[1] *Virchow's Archiv*, 1874, Bd. lix. S. 174; see also Ewald, *Ztschr. f. klin. Med.*, Berlin, 1887, Bd. xii. S. 407; Huber, *Deutsches Arch. f. klin. Med.*, Leipzig, 1891, Bd. xlvii. S. 495.

[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1879, S. 39.

during proteid digestion. Such evidence can only give a reliable estimate of the amount formed relatively to other proteid products, when the rate of absorption from the intestine of these various products is known.

Kölliker and Müller[1] found only microscopic traces of leucine and tyrosine in the upper part of the small intestine of carnivorous animals, and none in the lower part. Kühne[2] subjected fibrin to tryptic digestion in a tied-off loop of intestine, into which the pancreatic duct entered, and subjected the residue to analysis after four hours. Leucine and tyrosine were found among the products, but the yield was small, and, moreover, the conditions in such an experiment are not quite comparable to those of natural digestion. Indeed, Kühne himself thinks it probable that the greater part of the "peptone" being rapidly absorbed escapes such a decomposition.

Schmidt-Mülheim[3] states that, in proteid digestion in the carnivora, leucine and tyrosine are either not formed at all, or else in such small quantities that their absorption is of no physiological importance as a means of removing from the alimentary canal any appreciable amount of nitrogen derived from the proteid foodstuffs.

On the other hand, Sheridan Lea[4] obtained from the intestinal contents of a dog, six hours after a plentiful meal of lean flesh, what must be regarded as a considerable amount of amido-acids to be found there at any given time (particularly when it is remembered that the amount of intestinal contents in the dog at any given moment, even during active proteid digestion, is very scanty), namely, 1 grm. of pure leucine, and ·3 grm. of tyrosine. These figures equal the total amounts obtainable of these products from 10 grms. of dried proteid, and if it be assumed that leucine and tyrosine are absorbed with a rapidity equal to that with which albumoses are taken up, indicate that a considerable percentage of proteid was being converted into amido-acids, and absorbed as such.

The relative amount of proteid decomposed in the intestine into amido-acids, as well as that absorbed in the various other forms, probably varies within wide limits with the state of nutrition of the animal and the amount of proteid food. It is possible, as Foster[5] states, that such a degradation of proteid in the intestine may serve as a safety-valve to the economy, diverting from the tissues the burden of an often unnecessarily large proteid metabolism. The waste of energy to the animal economy caused by the disintegration of proteid into amido-acids in the intestine is often advanced as an argument against the occurrence of this process to any marked extent. Certainly the potential chemical energy of the proteid is lost to the economy, as far as the performance of some forms of physiological work is concerned, but it should not be forgotten that the *total* amount of energy abstracted by the animal from its food is measured by the chemical form in which it enters and that in which it leaves the body, and a given portion of proteid entering the body and then leaving it as urea, water, and carbon dioxide, will give up to the body exactly the same store of energy, no matter what may be the intermediate steps by which it is reduced from one form to the other. In one case the energy is set free in the tissues, in the other in the intestine. In the second case, the heat set free by chemical decomposition is communicated to the intestine and carried off by the circulating blood, to keep up the temperature of the body, thus sparing reserve chemical energy which would otherwise have to be used for this purpose.

[1] *Verhandl. d. phys.-med. Gesellsch. zu Würzburg*, 1856, Bd. vi. S. 499.
[2] *Virchow's Archiv*, 1867, Bd. xxxix. S. 130.
[3] *Loc. cit.*
[4] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 255.
[5] "Text-Book of Physiology," 1889, 5th edition, part ii. p. 476.

Changes in albumose and peptone during absorption.[1] — Although there is no doubt that a considerable, if not the greater part of the proteid of the food is absorbed as albumose or peptone, these bodies are never found in appreciable amount in the blood. Schmidt-Mülheim [2] stated that the maximum amount in serum is 0·028 per cent.; but recent experiments by Neumeister [3] have given an altogether negative result, and, according to this observer, albumoses are not present at all in blood, *even in traces.*

Injected directly into the blood, albumoses and peptone are treated by the organism as foreign bodies; they are not assimilable proteids, but are promptly excreted by the kidneys, unless injected in large quantities,[4] and in a short time practically all the peptone and albumose injected is found in the urine, while not a trace is to be found in the blood.[5] That albumose and peptone are foreign substances in the blood stream, is shown not only by this rapid elimination, but by the fact that they possess, besides, marked toxic properties, and cause the death of the animal when injected in larger doses, producing an immense and rapid fall in arterial blood pressure; in addition, they so alter the nature of the blood that on drawing it from the vessels it no longer coagulates, or does so very slowly. These results, taken in conjunction with the fact that normal urine never contains albumoses, even in traces, prove that the albumoses and peptones absorbed from the alimentary canal never reach the general circulation as such, but are somewhere on their route converted into other substances which can harmlessly enter the circulation. Positive experiments on the subject not only confirm this indirect proof, but clearly indicate that the change takes place in the lining epithelial cells.

Seat of the modification of albumose and peptone during absorption.— It might be supposed that the albumose and peptone disappeared as such in the liver; this is not, however, the case. Schmidt-Mülheim [6] found that the portal vein during proteid digestion contained no greater a percentage of these bodies than the arterial blood, and Neumeister [7] found that the portal vein, while absorption of peptone was going on, did not contain a trace of this material. Neumeister also circulated defibrinated blood, to which peptone had been added through a liver immediately

[1] In many of the papers referred to in this section, "peptone" is used to signify what would to-day be called a mixture of albumose and peptone; this has usually been translated by albumose and peptone, or by albumose.

[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 33. See also Hofmeister, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1885, Bd. xix. S. 17.

[3] *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 277. Neumeister caught the blood from the carotid in ammonium oxalate to prevent clotting; laked by shaking with ether; removed ether; saturated with ammonium sulphate; filtered; reduced filtrate by evaporating to a small bulk, filtering from time to time from crops of crystals; and tested in final filtrate for albumoses by the biuret test with negative results. Control experiments showed that even a trace of albumose added to the blood intentionally could be easily identified.

[4] When large amounts are injected, the fall in arterial blood pressure is so great that secretion of urine is arrested. Even in such a case the albumose does not remain in the blood, but passes into the lymph (Shore, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 549).

[5] Plóz and Gyergyai, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. x. S. 536; Hofmeister, *Ztschr. f. physiol. Chem.*, Strassburg, 1881, Bd. v. S. 131; Schmidt-Mülheim, *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 33; Fano, *ibid.*, 1881, S. 281; Shore, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 528. A similar effect follows subcutaneous injection (Hofmeister, *loc. cit.*).

[6] *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 33.

[7] *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1889, S. 65; *Ztschr. f. Biol.*, München, 1888, Bd. vi. S. 287.

after its removal from the body, and proved that the peptone remained unchanged. Also, when a small quantity of peptone or albumose was slowly injected into a mesenteric vein, this was not assimilated, but appeared afterwards in the urine, showing that it had not been altered by the liver. Shore [1] circulated peptone not only through the liver, but through the spleen also, by injecting into a splenic artery, and arrived at similar results; practically, all the peptone appeared again in the urine unchanged.

These results show that the albumose and peptone do not even enter the *portal* circulation as such; the only remaining place where they can undergo modification is in the wall of the intestine itself, and the following experiments show that this is the seat of change.

Ludwig and Salvioli [2] separated a loop of intestine in the dog with the attached portion of mesentery, and injected a gramme of albumose and peptone in 10 per cent. solution, ligaturing the piece of intestine at both ends. The piece of isolated intestine was maintained alive by circulating through it warm defibrinated blood diluted with normal saline, by means of a cannula inserted into that branch of the mesenteric artery which had supplied the loop (anastomosing arterial branches being excluded by ligatures), the blood, after circulating, flowing away by the corresponding branch of the mesenteric vein. After four hours of perfusion in this manner, the piece of intestine and the defibrinated blood having been all the time maintained at the body temperature, the remaining contents of the intestine and the circulating fluid were examined for albumose. The intestine contained about half a gramme of coagulable proteid, and only traces of albumose, while the defibrinated blood contained no albumose whatever. Therefore the albumose must have disappeared in the intestinal wall.

Hofmeister [3] investigated the organs of dogs killed during proteid digestion as to their content of albumose, and found it present in the mucosa (only) of the stomach and intestine, as well as in small quantities in the blood, and in four out of ten cases in the spleen; in the other organs it was entirely absent. He also showed experimentally that this albumose underwent a rapid change.

A fresh stomach was divided into two symmetrical halves, or a piece of small intestine longitudinally into two similar pieces.

The surface of the mucous membrane was washed clean with saline, then one of the two pieces in each case was thrown immediately into boiling water, while the other was similarly treated after being first kept for some time in a moist chamber at 40° C. More peptone was always found in the first piece than in the second, and when the second piece had been kept for a sufficient time (1 to 2 hours) at body temperature, previous to placing in boiling water, it was found to contain no albumose whatever. In another experiment, while one piece was thrown, as before, immediately into boiling water, the second was thrown for some minutes into water at 60° C., and then kept as before at 40° C. for two hours; the result now obtained was that both pieces contained an equal amount of albumose. Since most enzymes would not be affected by such a preliminary treatment, while living cells would be destroyed, this indicates that the cells of the mucosa do not owe their activity to contained enzymes.

[1] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 559; *Verhandl. d. X. internat. med. Cong.*, Berlin, 1891, Bd. ii. Abth. 2, S. 31.

[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, Supp. Band, S. 112.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 51; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1885, Bd. xix. S. 8.

Neumeister[1] states that albumoses and peptones dissolved in whipped blood can be changed by mere contact with pieces of living intestine, the rapidity of change being increased when a slow stream of air is driven through the mixture, so as to bring the pieces of intestine into rapid contact with different portions of the blood and albumose.

Hofmeister[2] observed a considerable increase in the number of leucocytes in the intestinal wall during digestion of proteids, and argued from this that these took a considerable share in proteid absorption and in the conversion of albumoses and peptones in the adenoid tissue of the intestinal wall, and in the mesenteric lymphatics. There is little experimental ground for belief in such a theory. In the first place, proteid is not absorbed to any appreciable extent by the lymphatics; secondly, albumoses are not changed, as Hofmeister[3] himself has shown, in the blood, which contains plenty of leucocytes; thirdly, Heidenhain[4] has shown that the amount of leucocytes in the wall of the intestine (and the amount of active mitosis in these) is too small to render them adequate for such a purpose. Finally, Shore[5] has shown that, after slow injection of a small amount of peptone (·049 grms.) into a lymphatic of the hind-limb in a dog, this can be detected again in the course of twenty minutes in the chyle flowing from a fistula of the thoracic duct, showing that it has traversed the lymphatic system unchanged.

All these experiments go to prove that albumoses and peptones are modified during their passage through the epithelial cells by the action of living protoplasm. What substances are formed from them is not known by direct experiment, but it is highly probable that the process is one of conversion backwards into coagulable proteid. It is known that coagulable proteid can be artificially obtained from peptone and albumose,[6] and that albumose and some forms of peptone used as foods can replace coagulable proteid in maintaining nitrogenous equilibrium. It is difficult to see how such a result can be attained otherwise than by a formation of coagulable proteid from albumose and peptone.

The percentage of any proteid foodstuff, which is absorbed from the alimentary canal, may be deduced fairly accurately from a comparison of the amount of nitrogen in the food with that of the urine and fæces when such a food is taken into the system.

Experiment shows that the various forms of proteid are utilised by the organism in widely varying degrees. It does not necessarily follow that a food of which the nitrogenous part is only partially absorbed is on that account to be despised as an adjunct to other classes of nitrogenous food; vegetable proteid is absorbed much more imperfectly than that from animal sources; but vegetable food, amongst other things, is valuable for the consistency and bulk it gives to the food,

[1] "Lehrbuch der physiol. Chem.," Jena, 1893, Th. 1, S. 251; *Ztschr. f. Biol.*, München, 1890, Bd. xxvii. S. 324.

[2] *Arch. f. exper. Path. u. Pharmakol.*, 1885, Bd. xix. S. 32; 1886, Bd. xx. S. 291; 1887, Bd. xxii. S. 306. See also Pohl, *ibid.*, 1888, Bd. xxv. S. 31; Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Supp. Heft., Bd. xliii. S. 72.

[3] Hofmeister (*loc. cit.*, Bd. xix.) is of the opinion that the portion of "peptone" which he believes enters the blood unchanged is converted in the tissue, "peptone" being found during digestion in the arteries but not in the veins. The presence of any albumose or peptone, even in the arteries, is, according to more recent observers, however, very doubtful.

[4] *Loc. cit.*

[5] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 553.

[6] See p. 400.

and for the mechanical stimulation its presence gives to the intestinal movements.

The small amount of vegetable proteid absorbed, compared with that of animal proteid, is in part due to the envelope of indigestible cellulose by which it is surrounded, in part to the shorter stay in the intestine due to its action in causing increased peristalsis, and in part to its own less digestible character.

The percentage of various kinds of plant proteid absorbed also varies considerably; thus the proteids of some leguminous plants and cereals are absorbed nearly as perfectly as those of animal origin, while in most others (potato, lentil) it is much less complete (22 to 48 per cent. less). The percentage of the nitrogen of meat or egg appearing again in the fæces in man, only amounts to 2·5 to 2·8 per cent., that of milk to 6 to 12 per cent.

Considerable tracts of the alimentary canal can be removed or thrown out of action without causing the death of the animal or even causing serious impairment in absorption.

The stomach was first removed by Czerny[1] in dogs; one animal was preserved alive after such an operation for five years; in the course of two months after the operation it recovered to quite a normal condition, and ate, digested, and absorbed all kinds of food. It was finally killed for examination by Ludwig and Ogata, and the dissection showed that only a very small portion of the cardiac end of the stomach remained.

Ludwig and Ogata[2] further investigated the course of digestion and absorption when gastric digestion is excluded, by another method. They made a fistula beyond the pylorus and inserted into the beginning of the duodenum a small thin rubber ball, attached to a rubber tube, by means of which it could be distended with water under pressure, so as to occlude the intestine from the stomach. In this way gastric juice could be prevented from entering the duodenum, and by feeding from the fistula the effect of intestinal digestion alone be studied. The food was usually completely digested and absorbed, and the fæces presented a normal appearance. Raw meat was digested much more efficiently than boiled, connective tissue was not so completely digested as in normal dogs, but nevertheless two injections of meat per diem sufficed to keep the animal in equilibrium.

The stomach has also recently been removed in dogs by F. de Fillipi,[3] who found no disturbance in metabolism and no increase in intestinal putrefaction in spite of the absence of hydrochloric acid.

The same experimenter also removed in a bitch 1·9 metres of the small intestine (almost the entire length), and found no metabolic disturbance, except that the absorption of fat was diminished; the animal lived, and afterwards brought up a litter of pups in this condition. The author suggests that the large intestine here vicariously took on the absorptive functions of the small intestine.

Complete or partial extirpation of the pancreas, or ligature of its duct, causes more or less disturbance of proteid digestion and absorption, but not so much as might be expected, in view of the most important proteolytic function of the secretion of this gland.

[1] "Beiträge z. operativen Chirurgie," Stuttgart, 1878, S. 141.
[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1883, S. 89.
[3] *Deutsche med. Wchnschr.*, Leipzig, 1894, No. 40, S. 780.

Minkowski and Abelmann[1] found, after complete removal of the gland in dogs, that on an average 44 per cent. of proteid was absorbed; after partial removal, 54 per cent. The amount of absorption was much increased on giving raw pancreas with the food. Sandmeyer[2] obtained similar results. On removal of all but one-fifth to one-fourth of the gland (the portion remaining behind not being in communication with the intestine), 60 to 70 per cent. of proteid was still absorbed, and, on adding a supply of finely-minced pancreas to the food, the absorption of proteid became almost normal.

DIGESTION AND ABSORPTION OF FATS.

The pancreatic juice is the only digestive secretion which contains an enzyme possessing a chemical action on the neutral fats.[3] This action consists in splitting the fats into fatty acids and glycerin,[4] and may be demonstrated in one of the following ways:—

1. A neutral fat is first obtained, *e.g.*, by thoroughly shaking olive oil with sodium carbonate solution and ether, pipetting off the ethereal layer, filtering if necessary, and finally allowing the ether to evaporate, when a neutral fat is left behind. This is mixed either with fresh pancreatic juice, or an extract of the fresh gland prepared as already described, and the mixture, after being coloured blue by the addition of litmus, is placed in a bath at 37° to 40° C. The alkaline reaction is seen gradually to change into an acid one.

2. Instead of adding litmus, after the mixture of neutral oil and pancreatic juice, or extract, has digested for some time (half to two hours), sodium carbonate solution is added (which converts the free fatty acids formed into soaps), and the unattacked fat is removed by repeated extraction with ether. The residue is next treated with dilute sulphuric acid, setting free again the fatty acids, which are extracted with fresh ether, and recovered after its removal by evaporation.

3. The formation of free fatty acid may be also qualitatively shown, by removing water from the fresh, finely-divided gland, with 90 per cent. alcohol, drying it with filter paper, and then covering it with a neutral ethereal solution of butter, obtained by shaking up milk or cream with ether and a solution of caustic soda. When this material is kept for a short time at 37° to 40° C., a distinct odour of butyric acid appears; and if the mixture has been previously rendered blue by litmus, this turns red.

Form in which fats are absorbed from the intestine.—There has been much discussion as to the *extent* to which the decomposition of the fats by the pancreatic enzyme, as above described, takes place in the intestine; and also as to the subsequent fate in the intestine of the fatty acids formed therein. According to the views held on

[1] "Ueber die Ausnutzung der Nahrungsstoffe nach Pancreasextirpation," Inaug. Diss., Dorpat, 1890; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1890, Bd. xx. S. 45.

[2] *Ztschr. f. Biol.*, München, 1895, Bd. xxxi. S. 35.

[3] Fats are said to undergo a certain amount of decomposition into fatty acids in the stomach (Marcet, *Proc. Roy. Soc. London*, 1858, vol. ix. p. 306; Cash, *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 323); the cause of this decomposition is unknown, but it is probably bacterial during the first stage of gastric digestion.

[4] Bernard, *Compt. rend. Acad. d. sc.*, Paris, tome xxviii.; *Arch. gén. de méd.*, Paris, 1849; "Mémoire sur le pancréas," Paris, 1856; "Leçons de physiologie expérimentale," tome ii. p. 256. For the chemical equations representing such a decomposition, see *Chemistry of the Fats*, p. 19.

these subjects by different experimenters, various theories have been propounded as to the form in which fats leave the intestine. These theories may be divided into two classes—(*a*) Those in which it is held that the fats are absorbed in particulate form, as emulsified fats or fatty acids; (*b*) those in which it is held that the fats are absorbed in solution as fatty acids or as soaps.

Emulsification.—All fat or oil which has not been specially neutralised contains a slight amount of free fatty acid. On long standing in contact with air, the amount of this fatty acid is increased, probably by bacterial action; when this proceeds beyond a certain limit, the fat is said to become rancid.

If such a rancid oil, or fat melted by gently warming, be briskly shaken up with a solution of an alkaline carbonate (*e.g.* a 0·25 per cent. solution of sodium carbonate), it becomes suspended permanently in the alkaline solution in the form of very minute particles or globules, and so forms what is known as a permanent emulsion. But if the rancid oil be previously carefully neutralised (*e.g.* by mechanically shaking for some hours with a saturated solution of barium hydrate at 95° C., and then pipetting off),[1] no amount of shaking with a solution of an alkaline carbonate afterwards will cause it to yield a permanent emulsion; the fluid on standing will quickly settle into two distinct layers. Neither can a lasting emulsion be obtained by shaking up a rancid oil or fat with distilled or acid water; some free fatty acid and some alkali must be simultaneously present. In other words, the necessary conditions for the formation of a soap must be satisfied.[2]

Emulsifying action of alkaline salts and bile.—Attention was first drawn to the action of alkaline salts in promoting emulsion by Marcet[3] in 1857; this author investigated the effect of both disodic phosphate and of bile on fatty acids and on neutral fats; his results have not obtained, even in English text-books, the attention they deserve, and seem in part to have become forgotten. The results with bile and fatty acids have an important bearing on more recent researches, to be subsequently described, and for this reason are here quoted at length.

Disodic phosphate, "when mixed with pure stearic and margaric acids prepared from sheep's fat, and heated, produced a perfect emulsion, resembling milk; on cooling, a substance solidified, consisting of fatty acids with more or less soda, soap, and a small quantity of phosphate of soda; therefore the formation of the emulsion had been attended with that of a small proportion of soap. When *neutral* fats were heated, suspended in a solution of phosphate of soda, *no emulsion occurred*; the fats fused, and, on cooling, solidified under the form of a hard cake; the warm mixture, although strongly shaken, was not converted into an emulsion, but the minutely divided globules of fat rose to the surface, uniting with each other, and solidified on cooling; the fluid remained perfectly clear.

"The next subject for inquiry was to determine whether *bile* exerts a similar action on fatty acids and neutral fats. On heating and agitating gently a mixture of fresh sheep's bile and fatty acid (margaric, stearic, and

[1] Rachford, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 73.

[2] Only formation of "artificial emulsions," if the expression may be used, from rancid oils is referred to here; it will be seen later that a pancreatic emulsion can be formed and persist in presence of an acid reaction due to fatty acids.

[3] *Compt. rend. Soc. de biol.*, Paris, 1857, p. 191; *Proc. Roy. Soc. London*, 1858, vol. ix. p. 306; *Med. Times and Gaz.*, London, 1858, N. S., vol. xvii. p. 209. The extracts are taken from the last quoted Journal.

oleic acids), prepared from sheep's fat, *as soon as the latter had begun fusing it disappeared, and finally the whole of the fatty acid was dissolved*; on standing, however, it was observed that a very few extremely minute globules of fat rose to the surface. As soon as the mixture had been allowed to become colder than the temperature of fusion of the fatty acids, it assumed a turbid appearance throughout, which gradually increased, the fluid becoming white and milky, slightly coloured by the bile; finally, if the fat present was in sufficient proportion, the whole mass was converted into a semifluid paste, possessed of a light green colour, and adhering so strongly to the sides of the vessel that it could be turned upside down without letting out its contents.

"On diluting this remarkable emulsion with water, its consistency only was altered, becoming thinner, but no decomposition occurred; on heating the diluted mass, the emulsion was dissolved; it disappeared, but no globules of fat could be seen floating on the surface beyond the few minute specks previously mentioned. Besides this physical action of bile on fatty acids, the phenomenon was accompanied by a chemical decomposition; for the bile, which was neutral or slightly alkaline before the experiment, had become strongly acid after being treated with the fatty acid.

"An experiment was now instituted to determine whether a similar phenomenon takes place when bile and *neutral* fats are mixed together. Indeed, it was hitherto generally admitted that bile had no action on neutral fats. The results of my observations confirm this view, for in no case could I succeed in obtaining an emulsion and chemical decomposition, by heating bile with pure sheep's fat or with oil, having a neutral reaction; on agitating the hot mixture the globules of fat were broken up, but on standing they rose to the surface, the bile being unaltered in its appearance and reaction. Consequently, bile exerts no action on neutral fats."

Since these experiments of Marcet, many observers have busied themselves with the nature and mode of formation of emulsions.[1]

Brücke found that the presence of a certain amount of free fatty acid was sufficient to emulsify the remaining neutral fat, and stated that the provision of a sufficient amount of free fatty acid to emulsify the rest was probably the chief function of the fat-splitting property of the pancreatic juice. He obtained emulsion of fats containing fatty acids with diluted egg albumin, with bile, and especially with solutions of sodium carbonate and of borax. Gad discovered spontaneous emulsion, and carried out exact experiments on the most favourable conditions for the formation of emulsions. A spontaneous emulsion means the formation of a permanent emulsion without any mechanical assistance by shaking; such as occurs when a drop of oil containing a sufficient percentage of free fatty acid (5–7 per cent.) is placed on an alkaline solution of suitable strength ($\frac{1}{4}$ per cent. sodium carbonate).

The following are the main conditions which influence the formation of spontaneous emulsions, according to Gad:—

1. The power of different fats to form emulsions by contact with the same fluid depends (*a*) on the amount of free fatty acid in the fat, (*b*) on the solubility of the soaps formed from these fatty acids, (*c*) on the viscosity of the fat.

2. The power of the same fat to form emulsions in contact with

[1] Kühne, "Physiol. Chem.," 1866, S. 129; Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1870, Bd. lxi. Abth. 2, S. 362; J. Steiner, *Arch. f. Anat. u. Physiol.*, Leipzig, 1874, S. 286; J. Gad, *ibid.*, 1878, S. 181; G. Quincke, *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix. S. 129; v. Frey, *Arch. f. Anat. u. Physiol.*, Leipzig, 1881, S. 382; Rachford, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 72.

different fluids depends (*a*) on the degree of alkalinity of the fluid, (*b*) on their chemical composition, in so far as this influences the solubility of the soaps formed.

3. The maximum of quantity and quality of emulsion formed coincides with those conditions under which no formation of a membrane can be demonstrated.[1]

There has been much discussion as to the factors at work in the formation and conservation of emulsions. Brücke was of the opinion that it was the dissolved soap which conferred on the solution the power of holding the globules apart, after they had been mechanically formed in it. Gad supposed that the breaking up of the globules into smaller ones was due to a want of correspondence of the rate of solution and diffusion of the soap formed into the outer solution with the rate of diffusion of fatty acids towards the surface of the globule. In case fatty acid diffuses from the inner part of the fat globule towards the common surface of oil globule and solution more quickly than it can be dissolved by the solution, a film or membrane of soap will form around the globule. This film will not form at all parts of the globule equally, and this will give rise to amœba-like movements (due to differences in surface tension).[2] Gad also supposes that the ultimate microscopic globules are surrounded by soap films which keep them from coalescing.

Quincke attributes the formation of the emulsion to the differences in surface tension produced by the formation of a soap solution round the globule; and he also assumes the existence of films of soap (solid or in solution) around the ultimate oil globules in the emulsion, which have the property of keeping the globules from coalescing.

There is no doubt that soap formation is an accompaniment to the formation of an emulsion of rancid oil in an alkaline solution, and it is easy to see how the formation of such a soap film, at accidentally varying rapidity, at different points on the surface of a globule of oil, will cause variations in surface tension at these points, and so cause the oil globule following the soap film which covers it, to be drawn out into various shapes and split up. Such surface tension phenomena may be observed when two liquids which mix, such as alcohol and water, are brought together. More mixing takes place at one point than another; as a consequence, the mutual surface tension is less at one point than another, and those rapid, streaming movements are produced which may always be observed when alcohol and water are mixed. Similarly, when a small piece of a substance like camphor is placed on water, rapid shooting movements take place, due to an accidentally unequal solution of the substance at different points in the circumference, and consequently varying surface tension, as a result of which the piece of camphor is rapidly moved about from place to place. In an exactly similar manner a globule of rancid oil will be pulled about, altered in shape, and broken up in an alkaline solution, from accidental variations in the strength of soap solution at different points on its surface, causing variations in surface tension and corresponding movements.

It is much more difficult to see how any *permanent* film of insoluble soap can be formed round the ultimate globules, or even a film of soap solution of different concentration from the rest of the menstruum in which the globules float. From Gad's conclusions, it should be observed that in the cases where emulsion takes place best and most quickly, no such soap film can be observed, so that this soap film cannot be experimentally demonstrated; it is merely a theoretical thing, devised from the supposed necessity of having something to keep the globules from coalescing. A proteid membrane surrounding the fat globules in milk was supposed to have a similar office, but microscopically or

[1] *Vide infra.*

[2] The words in parenthesis are added.

otherwise no such membrane can be demonstrated, and its existence is very doubtful.[1]

A cloud is an emulsion, an emulsion of water particles in air, and no one has ever supposed that the water particles are surrounded by membranes which keep them apart. The prevention of coalescence is the result of the action of several factors, of which our knowledge is not yet perfect. 1. One such factor is the magnitude of the suspended drops; the bigger the drops the more rapidly they will come together, and fall (or rise) out of solution.[2] The more mechanical agitation an emulsion is given, the longer it will persist under otherwise unfavourable circumstances. 2. Another factor is the viscosity of the menstruum; the greater this is the more slowly will the finely-divided globules be able to move through the fluid, under the influence of differences in specific gravity or mutual attraction, so as to pass out of solution or coalesce. 3. Another factor is the comparative specific gravities of the fat and menstruum. 4. Still another is the mutual surface tension between globule and menstruum; the greater this is, the greater will be the tendency to diminution of surface, and hence to coalescence. On the other hand, if the mutual surface tension were zero, the two fluids would mix in all proportions.

It has been objected, by those who believe in the existence of a film around the fat globules, to the contention that the altered nature of the menstruum is sufficient to account for the permanency of the emulsions obtained with fats and alkaline solutions, that a permanent emulsion cannot be obtained by shaking up neutral fat with a soap solution. But the conditions in the two cases are essentially different. Neutral fats and fatty acids mix together in a rancid fat or oil in all proportions. When such a mixture is submitted to the action of alkali, the soap formation takes place where the fatty acids are, that is, intimately mixed with the neutral fat. So that soap is formed everywhere at the surface of the mass, and, dissolving, carries away (in the surface tension diffusion streams above described) the intimately admixed fat from the main mass in a very finely subdivided condition. If the proper conditions exist in the solution, these minute fat particles will not coalesce again. Such a result is brought about by the viscosity and reduction in surface tension which the solution acquires by means of the dissolved soap. On the other hand, when neutral fat is shaken up with soap solution, no such disintegrating agency comes into action, and the only thing to replace it is the mechanical subdivision due to shaking. As v. Frey points out, the smaller the diameter of the fat globules, the greater is the mechanical force necessary to subdivide them; and it is probable that by no amount of agitation can so fine a subdivision be reached as is naturally attained by the formation of the soap amongst the fat. By very prolonged and vigorous agitation, v. Frey has obtained "mechanical emulsions" of very considerable stability, even with neutral fats and water. The very fine subdivision of the fat, and the increased viscosity of the menstruum occasioned by the dissolved soap, are hence quite sufficient to explain the permanency of emulsions of rancid oils and fats in alkaline solution.

Formation of emulsions in the intestine.—The formation of an emulsion of fats in the intestine was already known to Eberle[3] in 1834, but was first brought into prominence by the classic researches of Claude Bernard.[4] Bernard was unacquainted with our modern theories of the formation of emulsion, and did not associate this process with

[1] See v. Frey, *Arch. f. Anat. u. Physiol.*, Leipzig, 1881, S. 382; Soxhlet, *Landwirthsch. Versuchsstat.*, 1876, Bd. xix.

[2] See v. Frey, *loc. cit.*

[3] "Physiologie d. Verdauung," Würzburg, 1834.

[4] *Compt. rend. Acad. d. sc.*, Paris, 1849, tome xxviii. p. 249; *Arch. gén. de méd.*, Paris, 1849, Sér. 4, tome xix. p. 60; "Mémoire sur le pancréas," Paris, 1856.

the production of fatty acid by pancreatic juice, although he was the discoverer of this saponifying action. He states that, when neutral oil is shaken up with pancreatic juice, an *instantaneous* emulsion takes place; and, secondly, when neutral oil is submitted to the *prolonged* action of pancreatic juice, fatty acids are developed. Bernard considered the formation of emulsion in the intestine as a more important process than saponification, due to a ferment action, and speaks of a "*ferment emulsif.*" It is now certainly known that fatty acids are always formed in the intestine after the ingestion of fat, but an emulsive ferment is no longer believed in. The rapidity of fresh pancreatic juice in forming fatty acid is remarkable; thus Rachford,[1] in very favourable cases, found that a sufficient amount of fatty acid to form a spontaneous emulsion (5·5 per cent.) is formed in presence of bile and hydrochloric acid at room temperature in two minutes. This very rapid action explains the error into which Bernard fell.[2]

Pancreatic juice obtained from a permanent fistula has less emulsive power than that from a temporary fistula; it is also poorer in proteid, and, according to Kühne,[3] the emulsive power does not depend upon the alkali, for faintly acid juice is capable of producing emulsion. Minkowski is of the opinion that it is chiefly to the proteid that emulsion is due, basing his opinion on the observation, made by Abelmann[4] in his laboratory, that after excision of the pancreas no fat except that of milk is absorbed; unless minced pancreatic tissue be taken with the food, when other fats are also absorbed. These observations have been confirmed by Sandmeyer.[5]

Some observers[6] hold that emulsification does not occur at all inside the intestine, and others[7] state that a considerable amount of emulsification takes place, but that the granules of fat in the emulsion are not nearly so small as those found in the chyle.

Cash[8] found, in four experiments on dogs, that there was no emulsion in the intestine during active fat absorption. Moore and Rockwood,[9] in six out of sixteen experiments, obtained a similar result, but in the other ten experiments found emulsions in the intestine, containing fat globules of various dimensions, some of considerable size, but many exceedingly minute. These results indicate that in the dog at least, fats can be broken up and absorbed without undergoing previous emulsification. Still it should be borne in mind that these two different conditions of the intestine in the dog during fat absorption may be phases of the same process. The contents of the stomach are not discharged continuously into the duodenum, but from time to time the pyloric sphincter is relaxed, and a portion of the contents of the stomach ejected. It may well be that the condition of no emulsion is

[1] *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 92.

[2] The statement that the fat-splitting action of the pancreatic enzyme is very slow, and hence that probably only a small percentage of fat is so decomposed in the intestine (see Bunge, "Lehrbuch," Aufl. 3, S. 175), undoubtedly arises from most observers using not pancreatic juice but pancreatic extracts, in which the easily decomposable fat-splitting enzyme was only present in traces. Rachford's results with pancreatic juice clearly indicate that the pancreatic secretion is capable within the time of digestion of a fatty meal of decomposing all the fat into fatty acids and glycerin.

[3] "Lehrbuch d. physiol. Chem.," 1868, S. 122.

[4] Inaug. Diss., Dorpat, 1890.

[5] *Ztschr. f. Biol.*, München, 1895, Bd. xxxi. S. 40.

[6] Cash, *Arch. f. Anat. u. Physiol*, Leipzig, 1880, S. 323; Altmann and Krehl, *ibid.*, 1889, Anat. Abth., Supp. Bd. S. 86; 1890, Anat. Abth., S. 97.

[7] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Supp. Heft, Bd. xliii. S. 88. Other recent observers who describe an emulsion in the intestine are, Lebedeff, *Arch. f. Anat. u. Physiol.*, Leipzig, 1883, S. 504; Lewin, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 180.

[8] *Loc. cit.*

[9] *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 74.

that existing immediately after such a discharge from the stomach, while the emulsion condition is a later stage.

In whatever form fats may be absorbed from the intestine, it is certain that previous emulsification must greatly assist the digestive fluids, by exposing an infinitely greater surface to their action. It is also certain that in a great many cases, if not in all, previous emulsification does take place.

Emulsion theories of fat absorption.—It was for a long time a popular theory that only a small fraction of fat is split up in the intestine into fatty acid and glycerin; and that by means of the small amount of acid so formed, aided by that present in the fat as it leaves the stomach, the remainder of the fat is converted into a fine emulsion which passes as such into the villi, and reaches the central lacteal.[1] Such a statement may be found in most text-books, but the progress of recent work has had a tendency to cast grave doubts on its truth, and to show that, at least as a general statement, it is erroneous. The theory does not rest on any direct observation of the amount of fat which leaves the intestine as emulsified fat, compared with that which leaves it in other forms, such as soap, glycerin, and emulsified fatty acids, —such a direct observation, in the present state of our knowledge, is impossible,—but on indirect evidence, which is briefly as follows:—

1. The presence of a very small percentage of fatty acid is all that is necessary in presence of an alkaline solution to perfectly emulsify neutral fat.

2. This small amount of free fatty acid can readily be furnished by the action of the pancreatic enzyme even on neutral fats, and to aid this action all fats contain already some fatty acid mixed with them. The alkaline juices poured into the intestine are capable of supplying the alkali necessary for emulsification.

3. When an animal is killed during active fat digestion, the lacteals invariably contain a white milky emulsion, consisting mainly of neutral fats with a small percentage of alkaline soaps.

Therefore the most natural conclusion is that a fine emulsion is formed in the intestine which passes in some manner into the lacteal. The greater part of the fat is only physically, not chemically, altered in digestion, and passes through the whole process as a neutral fat.

The weak point in the emulsion theory of absorption always was, how the fat globules got into the interior of the villus and made their way to the lacteal. Although the fat granules in an emulsion are of microscopic dimensions, they are still large compared to the dissolved molecules of serum or egg albumin which are unable to pass into or out of the intestine through the epithelial cells. If fat granules pass into the epithelial cells at all, it must therefore be by means of a special kind of absorption in bulk by these cells, and not by a process even of selective diffusion from solution. Such an absorption by bulk is easily carried out by a cell of which the protoplasm is capable of free contraction, such as the amœba, or leucocyte, but it is difficult to conceive how it can take place with a fixed cell, such as those which line the intestine. Impressed, perhaps, with the necessity of some such protoplasmic movement, some observers have looked earnestly for proto-

[1] This theory was first stated by Brücke, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1870, Bd. lxi. Abth. 2, S. 362.

plasmic processes from the epithelial cells, and one or two [1] fancied they had discovered such appearances, but their observations have not been confirmed, and are undoubtedly erroneous. If the epithelial cells of the intestine possessed the power of absorbing in bulk fat granules, there is no obvious reason why other food particles, such as granules of starch or proteid, should not be similarly absorbed, but no such absorption has ever been observed, nor are they capable of absorbing finely subdivided granules of coloured matter, such as carmine.

The mucous membrane of the intestine contains an immense number of lymph corpuscles.[2] These are found not only in the lymphoid nodules, which occur so abundantly as solitary glands and Peyer's patches, but in the intestinal villi, even between the epithelial cells, where they may approach quite close to the free surface, and abundantly in the adenoid tissue underlying them. Now, such lymph corpuscles are capable of enveloping and so absorbing fat granules, and have been credited with an important function in the removal of fat from the intestine by so doing. It was stated by Zawarykin [3] that when fat absorption is going on, fat granules are to be found *only* in these lymphoid cells and not in the cells of the columnar epithelium. This statement is undoubtedly erroneous, for it is easy, from an animal killed after a meal rich in fats, to obtain sections showing the columnar cells filled with fat globules.

"During active fat absorption, especially if the amount of fat in the chyme is relatively large, the columnar epithelial cells become filled with globules of fatty matter. These globules are of variable size, and may occur in all parts of the cell, but they are generally largest in the part between the nucleus and the thickened border, and are often quite small near the attached end of the cell." [4]

It is evident, then, that the greater part of the fat, if not the whole of it, must be absorbed by the epithelial cells from the intestine. It is also very improbable that these cells take up the fat in the form of an emulsion. As has already been stated, the structure of the cell is unsuitable for such a function, and, in addition, fat granules have never been observed in the broad striated border. This almost amounts to a demonstration that the fat passes through the border of the cell in some soluble form, and is afterwards thrown down in a particulate form, as the result of a process of cell metabolism.

Emulsion theories of fat absorption are therefore being gradually replaced by theories of absorption in solution. These theories must next be discussed, but before doing so reference may be made to another emulsion theory of fat absorption introduced by Munk.

Theory of I. Munk. — Munk [5] showed that fatty acids can be emulsified under exactly the same conditions as rancid fats, and further that these fatty acids are capable of absorption, and can completely take the place in the animal economy of neutral fats, being in great measure

[1] v. Thanhoffer, *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. viii. S. 391; Fortunatow, *ibid.*, 1877, Bd. xiv. S. 285.

[2] These wandering cells (*Wanderzellen*) were first described as occurring in the epithelium by Eberth (*Würzb. med. Ztschr.*, 1864, S. 23); Arnstein (*Virchow's Archiv*, 1867, Bd. xxxix. S. 537) first mentioned the presence of fat granules in them.

[3] *Arch. f. d. ges. Physiol.*, Bonn. 1883, Bd. xxxi. S. 231.

[4] Schäfer, *Internat. Monatschr. f. Anat. u. Histol.*, Leipzig, 1885, Bd. ii. S. 6.

[5] *Verhandl. d. Berl. med. Gesellsch.*, March 1879; *Arch. f. Anat. u. Physiol.*, Leipzig, 1879, S. 371; *Virchow's Archiv*, 1880, Bd. lxxx. S. 10; *ibid.*, 1884, Bd. xcv. S. 409; *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 568; *Arch. f. Anat. u. Physiol.*, Leipzig, 1890, Supp. Bd., S. 138. See also v. Walther, *ibid.*, 1890, S. 329.

converted into fats somewhere on their way from the intestine to the thoracic duct. He is hence of the opinion that in the normal course of digestion a considerable but indeterminate amount of fat may be absorbed in the form of emulsified fatty acids.

Munk's experimental results as to the absorption and synthesis during the process of absorption of the fatty acids, are of the highest importance; but it in no wise follows from them that the fatty acids are absorbed in the form of an emulsion. Such a theory is subject to the same objections as have above been urged against the older theory of absorption as emulsified fats. The fatty acids are probably taken up from the intestine by the epithelial cells in some *soluble form*, and synthesised to neutral fats in these cells.

Solution theories of fat absorption.—*Theory of absorption as soaps.*—One of the most important theories of fat absorption in soluble form is, that the neutral fats are split up by the action of the pancreatic enzyme into fatty acids and glycerin, that the fatty acids unite with a part of the alkali of the intestinal secretions to form alkaline soaps which are soluble in water, and that the alkaline soaps and glycerin are absorbed in solution by the epithelial cells, and there synthesised back to neutral fats. This theory is supported by a good deal of experimental evidence. Radziejewski[1] showed that alkaline soaps were absorbed; Perewoznikoff,[2] that a mixture of alkaline soap and glycerin was absorbed and synthesised to neutral fat. The lacteals had the usual milky appearance seen after a fatty meal; microscopic preparations, stained with osmic acid and with alkanna, showed in the tissue of the villi, *and in the epithelial cells*, fat globules of varying size. Will,[3] working under Grünhagen's direction, confirmed these results by histological observations on the frog; further, he showed that the presence of glycerin was unnecessary. Will made two kinds of experiments. In one he fed the frogs, which had previously been deprived of food, with the materials to be tested; in the other, he injected the materials into the living but cut out intestine, and then examined teased specimens stained with osmic acid. In both series the same results were obtained, on feeding with a mixture of pure palmitic acid and glycerin, or of potassium palmitate and glycerin; at the end of twenty-four hours an examination of the villi showed a formation of fat, by the presence everywhere of large distinct fat globules. Injection of palmitic acid alone into the intestine also led to the appearance of fat globules in the epithelium,[4] but these were not nearly so numerous as in the cases in which the palmitic acid was mixed with glycerin. As Salkowski and Munk had shown that fatty acids can be emulsified under certain conditions,[5] Will proceeds to show that this could not be the case in his experiments, and that the fat globules blackening with osmic acid in the epithelial cells are not *free* fatty acid. The free fatty acids only become emulsified when melted, and as pure palmitic acid

[1] *Virchow's Archiv*, 1868, Bd. xliii. S. 271; 1872, Bd. lvi. S. 211.

[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1876, S. 851.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xx. S. 255. See also v. Krehl, *Arch. f. Anat. u. Physiol.*, Leipzig, 1890, Anat. Abth., S. 97.

[4] In thus showing the formation of fat from fatty acid alone, Will anticipated I. Munk, but to Munk belongs the merit of clearly showing from the chemical standpoint that the organism, probably the epithelial cells, can furnish the glycerin radicle for the synthesis of neutral fats from the fatty acids.

[5] *Sitzungsb. d. Berl. physiol. Gesellsch.*, March 1879; *Virchow's Archiv*, 1880, Bd. lxxx. This was a re-discovery of a fact known to Marcet many years previously, see p. 444.

only melts at 62° C., such a thing could not occur in the frog's intestine. Moreover, a microscopic examination of the intestinal contents at the end of an experiment showed only amorphous masses of fatty acid and no emulsified globules. Will concludes that the fatty acid must be absorbed as a soap and not as an emulsion.

That the mucous membrane of the small intestine is capable of taking part in such a synthetical process, is shown by experiments of Ewald,[1] who dried the mucous membrane of a dog's intestine, which had been killed in a condition of hunger, at a low temperature after the method introduced by Brown and Heron,[2] and showed that this was capable of inducing the formation of neutral fat, from a mixture in proper proportions of soap and glycerin.

This experiment shows that, provided glycerin and soap are formed in the intestine, there is an agency provided for synthesising them back into neutral fats. Let us next consider what the probabilities are that such a complete decomposition, into fatty acids and glycerin followed by solution of the fatty acids as alkaline soaps, takes place in the intestine.

The idea that only a small fraction of the fats is decomposed in the alimentary canal into fatty acids and glycerin, has arisen from repetition of the emulsion theory only, and not from any experimental observation of lack of intensity of action of the fat-splitting ferment. Hoppe-Seyler[3] found that most of the fatty matter in both small and large intestine was composed of stearic and palmitic acids accompanied by very little neutral fat, and concludes that the decomposition into the fatty acids and glycerin is much greater than is usually supposed. Rachford[4] states that pancreatic juice must act very rapidly on fats, under the favourable conditions found in the duodenum, and is capable, unless checked or retarded in some manner, of splitting all the fats of the food into fatty acids and glycerin in the time required for intestinal digestion.

It may be concluded, then, that there is sufficient fat-splitting power provided in the intestine for the complete conversion of the fats into fatty acids; and it has been already pointed out that, on feeding with fatty acids or with soaps, these are absorbed, and converted into fats in the process. It only remains to consider, in connection with the soap theory, whether, in the natural process of fat digestion and absorption, it is probable that the fatty acids so set free combine with alkalies to form soaps, or whether they are absorbed in some other soluble form.

It has been objected to the theory of absorption in the form of soaps, that the reaction of the small intestine in the dog during fat absorption is not alkaline, but acid; that soaps cannot persist in presence of an acid reaction, and hence that fats cannot be absorbed as soaps.

Cash[5] investigated the reaction of the intestine in three experiments on dogs, in which the animals were fed on a mixture of starch and fat, and in three similar experiments in which the animals were fed on fat alone. He

[1] *Arch. f. Anat. u. Physiol.*, Leipzig, 1883, Supp. Bd., "Festschrift f. du Bois-Reymond," S. 302, Vorläufige Mittheilung.

[2] *Proc. Roy. Soc. London*, 1880, p. 393.

[3] *Virchow's Archiv*, 1863, Bd. xxvi. S. 534, Anmerkung.

[4] *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 92.

[5] *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 323.

found the reaction of the intestinal contents to be acid all the way from pylorus to cæcum.

Vaughan Harley[1] tested the reaction of the upper and lower halves of the small intestine, and of the large intestine, in three dogs which had been fed on milk, and found that the reaction was acid in all three portions.

Moore and Rockwood[2] have recently studied the reaction of the intestine in the dog during fat absorption to different indicators, chosen with a view to determining, not only the reaction, but also the character of the acids or bases causing that reaction. The indicators used were litmus solution, methyl-orange, and phenolphthalëin. The reaction to litmus of the upper part of the small intestine was found to be acid, changing to alkaline at a somewhat variable point, situate two-thirds to three-fourths of the way from pylorus to cæcum. The contents of the large intestine are commonly acid to litmus, while the reaction of the contents of the cæcum lies intermediate between that of the contents of the ileum and that of the contents of the large intestine, and may be either faintly alkaline or faintly acid.

The reaction at the pylorus, and for some distance below, may be nearly neutral or even faintly akaline to litmus; as the distance below the pylorus is increased, the reaction always becomes more strongly acid at first, then less acid again, and finally faintly alkaline at the limit described above. On testing with the other two indicators, it was found that the reaction was invariably alkaline all the way to methyl-orange, and acid all the way to phenolphthalëin.

These results seem at first sight confusing and contradictory, yet a consideration of the properties of the indicators used, not only renders them intelligible, but gives an indication as to the nature of the substances to which the contents of the intestine during fat absorption owe their reactions. An organic indicator only reacts to an acid which is stronger than the acid which it itself contains in its molecule; to a weaker acid it is stable, and hence shows no acid reaction; and in case the weaker acid is present as a salt, it decomposes that salt and reacts to the base with which it was combined, giving an alkaline reaction. Now, methyl-orange is a very stable, phenolphthalëin a very unstable, indicator, while litmus lies intermediate between these two. Methyl-orange reacts sharply to the inorganic acids, less so to the stronger organic acids such as acetic acid, and not at all to carbonic acid and the weaker organic acids, including stearic, palmitic, and oleic acids. With alkaline salts of these weaker acids (carbonates, bicarbonates, and the soaps) it gives an alkaline reaction. Phenolphthalëin reacts to traces of the weakest organic acids, and to carbonic acid; to normal sodium carbonate it is alkaline; to sodium bicarbonate, neutral; with excess of carbonic acid, acid. Litmus reacts to even weak organic acids, but the reaction is feeble, and a considerable excess is necessary to give a clear reaction; to carbonates and bicarbonates of the alkalies, it is alkaline.

These considerations make it evident that the acid reaction of the upper part of the small intestine to litmus during fat absorption is due to weak organic acids, probably to dissolved acids set free from fats;[3] while the alkaline reaction to methyl-orange can only be due to weak organic acids combined with alkalies, *i.e.* in all probability to dissolved soaps.

Since the acid reaction of the intestine during fat absorption is due to weak organic acids, the contention that soaps cannot be present falls to the ground. For the soaps would not be decomposed by these acids.

An objection, and apparently at first sight a very serious one, to

[1] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 2.
[2] *Ibid.*, 1897, vol. xxi. p. 58.
[3] *Vide infra.*

absorption in the form of soaps, is that urged by I. Munk, namely, the enormous quantity of alkali which would be required for such a purpose. Munk[1] reckons that to so combine with the fatty acids of 200 grms. of fat, about 40 grms. of sodium carbonate (Na_2CO_3) would be required. Now a dog of 25 kilos. can easily digest from 200 to 350 grms. of fat in twenty-four hours.[2] Supposing only 200 grms. are digested, and that all this is absorbed as soaps and glycerin, about 40 grms. of sodium carbonate will be required for the purpose; now the total blood only contains, in such an animal, alkali equivalent to 6 grms. of Na_2CO_3; if the other fluids of the body be supposed to contain an amount of alkali equivalent to another 6 grms. of sodium carbonate, the total alkalinity is equivalent to that of 12 grms. of sodium carbonate.[3] Therefore, to suffice for the absorption of the fatty acids as soaps, from three to four times the total alkali of the body must pass out in the intestinal secretions, and be reabsorbed with the fatty acids, during twenty-four hours. This is obviously impossible; therefore the fats are not absorbed as soaps and glycerin.

This objection of Munk loses, however, most of its weight, when the probable processes taking place, in case fats are absorbed as soaps and glycerin, and synthesised again to neutral fats in the epithelial cells, are carefully considered. In the synthesis of fat from soap and glycerin within the cell, alkali is again set free in exactly equal amount to that in which it was used up in the intestine, and this alkali must be got rid of by the cell in some manner. Why should it not be sent back again into the intestine, and act as a carrier to a fresh quantity of fatty acid as soap into the cell? In such a fashion a very small amount of alkali would suffice to explain the carriage of all the 200 grms. of fat as dissolved soap and dissolved glycerin into the epithelial cells.

It might possibly be further objected that soaps are only present in small quantity in the intestinal contents. But this applies also to alkali albumin, propeptones, peptones, and sugars; in fact, to all the products of the digestion of both proteids and carbohydrates. If soaps are normally absorbed by the epithelial cells, it is probable that these cells possess a selective capacity for soap absorption, as they do for many other products of digestion, and hence that the soaps are absorbed as they are formed, and never allowed to accumulate in appreciable quantity in the intestine.

There is, then, no proof that soaps cannot be formed in the intestine, nor is there any impossibility or improbability in the way of all the fats being first decomposed into fatty acids, then converted into soluble soaps and absorbed as such.

Theory of absorption as dissolved fatty acids.—Another theory is, that the fats are absorbed in the form of dissolved fatty acids.

The fatty acids of the fats are practically insoluble in water, but are soluble to a certain extent in bile, the solubility increasing with rising temperature. Strecker[4] stated, in 1848, that taurocholic acid possesses the property of dissolving fat, fatty acids, and cholesterin in considerable quantity. This fact is mentioned by Strecker in connection with the difficulties attending the

[1] *Virchow's Archiv*, 1880, Bd. lxxx. S. 11; 1884, Bd. xcv. S. 408.

[2] Pettenkofer and Voit, *Ztschr. f. Biol.*, München, 1873, Bd. ix. S. 30.

[3] These figures must only be taken as argumentative data, overstepping the truth, and not as truly indicating the total alkalinity.

[4] *Ann. d. Chem.*, Leipzig, 1848, Bd. lxv. S. 29.

preparation of taurocholic acid in a pure condition from bile. He did not pursue the subject further on its own account, and his statement is in part erroneous, for the neutral fats scarcely dissolve at all in bile. In 1858, Marcet[1] published the results already described, showing the great solubility of the fatty acids in bile when heated above their melting points. Latschinoff[2] described a variable compound, or rather mixture, formed by taurocholic acid with a mixture of stearic and palmitic acids, which possesses certain crystallographic properties, but no definite chemical composition.

Altmann,[3] mainly on histological grounds, concluded that fats are not absorbed as an emulsion, but in some soluble form.

Krehl,[4] under Altmann's direction, obtained sections of the intestine, stained by osmic acid, from animals killed at varying times after feeding on fat (olive oil and cream). These preparations showed a gradual increase in the size of the globules with the advancement of the period of digestion. Also, it was observed that in the earlier stages the small fat globules showed a clear centre, surrounded by a dark ring. From these appearances it was judged that the formation of the fat granules was a gradual one from solution, and not from drops of fat emulsion. In considering the soluble form in which the fats are absorbed, Altmann rejects the idea that they are absorbed as soaps, chiefly on the ground that the reaction in the small intestine of the dog is acid, so that it cannot contain dissolved soaps;[5] yet from such a portion of intestine, with an acid reaction and containing a clear fluid, the charged lacteals are often to be seen conveying away absorbed fat. Altmann cites the statements as to the solubility of the fatty acids in bile already mentioned,[6] and adds an experiment of his own, in which he shows that a considerable, but not too great, quantity of a solution of commercial glycerin soap, and then excess of hydrochloric acid, may be added to a solution of sodium glycocholate or taurocholate without producing any precipitation of either fatty or bile acid. From these data, and the observation of Munk that the fatty material found in the dog's intestine during fat digestion may contain as much as 12 per cent. of free fatty acids, Altmann argues that the free fatty acids are dissolved in the intestine by the bile acids. As the fatty acids so dissolved are absorbed, fresh amounts of the neutral fats are decomposed, and the free fatty acids so formed pass into solution to replace those removed by absorption. So that there is a cyclic process involving the decomposition of fats, solution of fatty acids in the bile acids present, absorption of these fatty acids by the intestinal cell, and regeneration of neutral fat within the cell, accompanied by the appearance of fat granules.

Altmann did not quantitatively determine the amount of solubility of fatty acids in bile acids, bile, or intestinal fluid. The solubility in bile varies greatly with temperature, as is shown by Marcet's experiments.[7] At the temperature of the body the solubility is much less than at the temperature of fusion of the fatty acids, but is still considerable; while at ordinary atmospheric temperature (14° to 15° C.) the solubility is very slight.[8]

The solubilities of the fatty acids, and mixtures of these at or near the

[1] See p. 444.
[2] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1880, Bd. xiii. S. 1911.
[3] *Arch. f. Anat. u. Physiol.*, Leipzig, 1889, Anat. Abth., Supp. Bd. S. 86.
[4] *Ibid.*, 1890, Anat. Abth., S. 97.
[5] This objection is discussed under the soap-absorption theory. See p. 453.
[6] Except those of Marcet. [7] See p. 444.
[8] Moore and Rockwood, *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 58.

temperature of the body, have recently been determined by Moore and Rockwood,[1] in the bile of the ox, pig, and dog, and in the mixed bile salts of ox bile, with the following results :—

1. Pure palmitic and stearic acids are practically insoluble in ox bile at a temperature of 38° to 40° C., while oleic acid is easily soluble at this temperature to the extent of 4 per cent.

2. Of the mixed fatty acids of lard, beef-suet, and mutton-suet, respectively, lard acids are most soluble, mutton-suet acids least soluble, while beef-suet acids are intermediate. Thus in ox bile the solubilities are—lard fatty acids, 3·5 per cent.; beef-suet fatty acids, 2·5 per cent.; mutton-suet fatty acids, 2 per cent.

3. The solubility of the fatty acids in bile is only in part due to the bile salts. A strong solution (9 per cent.) of the bile salts of ox bile dissolves all three mixtures of fatty acids both more feebly and more slowly than bile itself. Mere removal of the "pseudo-mucin" from bile greatly diminishes its solvent action on fatty acids.

The same experimenters have shown that the filtered contents of the dog's intestine, removed during fat absorption, are capable, in some samples, of digesting and dissolving at body temperature to a clear solution as much as 4 per cent. of neutral fats. On cooling, the dissolved fatty material was thrown out of solution as fatty acids. This experiment shows that, in the dog at least,[2] fats can be dissolved and absorbed in solution as fatty acids.

The solubilities of the mixed fatty acids in bile, stated above, are quite sufficient to account for the absorption of all the fats of the food in the form of dissolved fatty acids, since they exceed the concentrations in which the products of carbohydrate and proteid digestion are met with in the intestine. But this alone is not sufficient evidence to prove that in the normal course of events all the fat is absorbed in such form.

The acids of the fats give an acid reaction with litmus. The bile used in the experiments arranged to determine the solubilities was at first strongly alkaline to litmus, but after it had dissolved the fatty acids it became markedly acid to that indicator. It follows, that a fluid with an alkaline reaction to litmus cannot hold in solution any free fatty acids. Now, in the intestine of the white rat, during active fat absorption, the reaction is commonly strongly alkaline to litmus, all the way from pylorus to cæcum, and is never acid to that indicator for a greater distance than 6 in. from the pylorus.[3]

Further, even in the case of the dog, and in that part of the intestine where the reaction is acid to litmus, there are probably soaps as well as fatty acids in solution. This is shown by the behaviour towards litmus and methyl-orange of the contents of this part of the intestine. The acid reaction towards litmus is shown by the alkaline reaction to methyl-orange to be due to very weak organic acids; at the same time, the alkaline reaction to methyl-orange also shows that there is an excess of bases present (above the amount necessary to combine with the strong acids), which is combined with very weak acids. The most probable conclusion, as such a state of affairs is met with during the digestion of an almost purely fatty meal (beef-suet), is that these weak acids are the acids of the fats (oleic, palmitic, and stearic) in combination as soaps. Hence, in that part of the small intestine of the dog where the reaction is acid to litmus, fat absorption is probably going on, partly in the form of dissolved fatty acids and partly in the form of dissolved soaps; in the part where the reaction is alkaline to litmus, wholly in the form of dissolved soaps.

In those animals, such as herbivora, in which the reaction of the intestinal contents is strongly alkaline, it is probable that all the fat is absorbed as soaps.

[1] *Loc. cit.*

[2] Similar results were not obtained with filtered intestinal contents obtained from the rabbit or pig.

[3] Moore and Rockwood, *loc. cit.*

If a rabbit be killed some hours after a meal of oats, a certain amount of fat is shown to be in process of absorption by the whiteness of the lacteals, but the reaction of the contents of the small intestine is always markedly alkaline.

It is probable, then, that in all animals a great part of the fat is absorbed dissolved in the form of soaps; but in some animals a part is also absorbed as dissolved fatty acids, while in others the entire quantity leaves the intestine in the form of soaps.

Fat absorption

These various theories as to the form in which fats enter the epithelial cell, may be summarised as follows:—

Emulsion theories.—1. A small percentage of the fat is split up into fatty acids and glycerin, the fatty acids unite with the alkaline basis of the mixed secretions present in the intestine, and the rest of the fat is thereby converted into an emulsion, which is absorbed by the columnar cells.

2. A considerable part of the fat is split up into fatty acids and glycerin, and absorbed as *emulsified* fatty acids and glycerin, which are synthesised to neutral fats by the columnar cells.

Solution theories.—1. All the fat is split up into fatty acids and glycerin; the fatty acids combine with alkaline bases to form soluble soaps; these and the dissolved glycerin are absorbed in solution, and synthesised to neutral fats in the columnar cells.

2. All the fat is split up into fatty acids and glycerin; the fatty acids are dissolved as such by the intestinal fluid (the bile being that constituent which gives this solvent property to the fluid), these dissolved fractions of the fat are absorbed by the columnar cells, and by these are synthesised again to neutral fats.

3. The processes indicated under solution theories 2 and 3 probably mutually replace each other to a variable extent in some animals, but in others absorption takes place entirely in the form of soaps.

Passage of the fat from the epithelial cells to the lacteals.—In whatever form the fat passes into the columnar cells, it is certain that it is here converted again into fat. During active fat absorption these cells become gorged with fat globules of varying dimensions. It is agreed by all observers that this fat passes from the epithelium to the lacteals in the form of an emulsion, but there is some difference of opinion as to the fashion in which it is conveyed.

It has already been stated that the tissue of the villi, especially during active fat absorption, contains immense numbers of leucocytes. These are found not only in the subepithelial tissue, but between the epithelial cells. The number in this position is greatly increased during absorption, and at this time lymphoid cells occur also in the lacteals, but "are found more numerously in the lacteals of the villi than in those which are more deeply seated, and, most numerously of all, near the blind end of the lacteal. That they pass into this vessel from the surrounding lymphoid tissue is certain, for a lymphoid cell may often be seen, fixed by the reagent employed for hardening the tissue, in the act of passing through the wall of the lacteal."[1] After a meal containing fat, these lymphoid corpuscles contain granules, which stain black with osmic acid; many of these are soluble in ether, so that they are unquestionably composed of fat.

[1] Schäfer, *Internat. Monatschr. f. Anat. u. Histol.*, Leipzig, 1885, Bd. ii. S. 6. The greater part of the description of the carriage of fat by leucocytes, between epithelium and lacteal, given in the text, is abstracted from this source.

These appearances led Schäfer[1] to express the view that the lymphoid corpuscles have an important function in taking up the fat from the epithelial cells, and carrying it towards and into the lacteal, where they set the fat free by disintegrating. No fat particles are, as a rule, found between the epithelium and the central lacteal, save such as are embedded in lymphoid corpuscles. Nor is there any channel of communication between the epithelial cell and the lacteal, as was formerly supposed, by which the fat globules might be carried into the lacteal. The epithelial cells never penetrate the basement membrane, nor are they continued into the cells of the retiform tissue beneath. Wiemar[2] admits the presence, during fat absorption, of fat granules in the leucocytes, but from the small amount of fat so found, compared with that in the epithelial cells, considers that the leucocytes can only be of secondary importance. In this connection it should be noted that Schäfer[3] has pointed out that the relative amount of fat granules in leucocytes and epithelial cells varies with the activity of absorption. "When the absorptive activity is feeble, or when the amount of fat in the chyme is relatively small, there may be little or no fat in the columnar epithelial cells, although the amœboid cells between them may be gorged with fat granules. In frogs fed with lard in the spring, fatty globules are still abundant in the columnar epithelial cells on the eighth day after the feeding, whereas, in frogs similarly fed in November, the greater part of the fat was discharged *per anum*, by the third day, very little being absorbed, and what was being taken up during that time was only to be found in the amœboid cells, none at all being present in the epithelial cells themselves." This seems to indicate that, when the rate of absorption is slow, the amœboid cells are able to keep pace with it, but when the supply is too abundant for this, the columnar cells act as temporary storehouses, and become filled with granules, which are afterwards carried off by the amœboid cells.

Heidenhain[4] ascribes only a secondary importance to the leucocytes. He gives as grounds for this opinion—(1) That in newly-born puppies, which have already sucked, and in which milk absorption is going on, there are scarcely any leucocytes present in the epithelium, so that there is no constant connection between fat absorption and the presence of leucocytes. (2) Leucocytes containing granules, which stain black with osmic acid, are to be found in the crypts of Lieberkühn, into which fat cannot enter from the intestine. (3) The material which is stained black with osmic acid is chiefly something else than fat, since it stains with acid-fuchsin, and cannot be washed out of adhesively mounted sections by ether or xylol.[5] Heidenhain[6] admits, however, that in the guinea-pig fat is undoubtedly present in considerable quantity in the amœboid cells during fat absorption.

Heidenhain[7] still adheres to the emulsion theory of absorption, but

[1] Quain's "Anatomy," 8th edition, 1876, vol. ii. p. 363; "Pract. Histology," 1876, p. 194; *Internat. Monatschr. f. Anat. u. Histol.*, Leipzig, 1885, Bd. ii. S. 6; *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiii. S. 513. Schäfer's observations were chiefly made upon the frog and rat.

[2] *Ibid.*, Bd. xxxiii. S. 532.

[3] *Internat. Monatschr. f. Anat. u. Histol.*, Leipzig, 1885, Bd. ii. S. 6.

[4] *Arch f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. Supp. Heft, S. 82.

[5] It should, however, be pointed out, that after prolonged treatment with osmic acid, fats tend to become insoluble in these fluids.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii., Supp. Heft, S. 103, figs. 39 and 40, plate iv.

[7] *Ibid.*, S. 88.

offers no explanation of the mode in which the fat granules get into the epithelial cells; he considers that the bile must essentially assist in the process, partially by aiding the emulsification of the fats, and partially by making the surface of the epithelium capable of being wetted by the fats, which naturally facilitates the absorption. He is further of opinion that the fat globules are passed on out of the columnar epithelial cells by means of the contractions of the cell protoplasm; and that these on their further path to the lacteal, apart from the small part eaten up by the leucocytes, pass in a free condition through the intercellular spaces, and are first broken up into the very fine granules characteristic of chyle when passing into the lacteal.

The effects of absence of the pancreatic juice or bile on the absorption of fats.—The results on record as to the absorption of fat, when the action of the pancreatic juice is removed by excision of the pancreas, ligature of the pancreatic ducts, or establishment of a pancreatic fistula, vary considerably; although there is a concurrence of opinion amongst recent observers [1] that the absorption of fat is hindered to a greater or lesser extent by the absence of the secretion. Minkowski [2] and Abelmann [3] found that no fat, except that of milk, was absorbed after complete removal of the pancreas, and this was only absorbed to the extent of 28 to 53 per cent.; the failure of absorption was not due to absence of fatty acids, for 80 per cent. of the ether extract of the fæces was found to be free fatty acid.

Minkowski believes that the absorption of the milk fat is due to this emulsion being able to withstand an acid reaction, but the absorption of other fats, when pancreas is given with the fat, points rather to some specific function of the pancreatic juice, for this pancreatic tissue could not materially alter the reaction of the intestine; besides, fat absorption takes place normally from the dog's intestine in presence of an acid reaction. Sandmeyer [4] found in dogs in which the pancreas had been partially extirpated, that the amount of fat absorption was very variable; occasionally no fat at all was absorbed, and at other times, with the same animal, 30 and even 78 per cent. of the fat was absorbed.

Teichmann [5] found by microscopic examination that fat absorption in the rabbit was not influenced to any marked extent by ligature of the pancreatic duct. Fr. Müller [6] observed a considerable amount of fat absorption in a patient with a pancreatic fistula. Vaughan Harley [7] extirpated the pancreas completely in dogs, killed the animals a varying number of hours after feeding on milk,

[1] On the other hand, Cohn (*Bull. Acad. de méd.*, Paris, 1856) found that the absorption of fat was not affected when the pancreatic juice was allowed to escape from a fistula. Cash (*Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 323) ligatured both pancreatic ducts in the dog, and found that fat was still absorbed. Schiff (*Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, Bd. ii. S. 222) shut out the pancreatic secretion by injecting paraffin into the duct, and found that fat to the amount of 120 to 150 grms. per diem was still absorbed.

[2] Von Mering and Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1890, Bd. xxvi. S. 371.

[3] Inaug. Diss., Dorpat, 1890 ; Minkowski, *Berl. klin. Wchnschr.*, 1890, S. 333.

[4] *Ztschr. f. Biol.*, München, 1895, Bd. xxxi. S. 12. See also Rosenberg, *Arch. f. Anat. u. Physiol.*, Leipzig, 1896, Physiol. Abth., S. 535.

[5] "Mikroskop. Beitr. z. Lehre von der Fettresorption," Diss., Breslau, 1891.

[6] *Ztschr. f. klin. Med.*, Berlin, 1887, Bd. xii. S. 45. Defective fat absorption, however, undoubtedly accompanies disease of the pancreas, or occlusion of its duct in most cases; see Bright, *Med.-Chir. Trans.*, London, 1832 ; Ziehl, *Deutsche med. Wchnschr.*, Leipzig, 1883, S. 538 ; le Nobel, *Deutsches Arch. f. klin. Med.*, Leipzig, 1888, Bd. xliii. S. 285.

[7] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 1.

and estimated the amount of fatty material in the stomach and intestine. The amount so found was usually slightly in excess of that given in the food, the surplus being probably due to intestinal secretion or excretion. Lewin,[1] as a result of microscopic examination of sections of the intestine, concludes that fat absorption does not take place in a normal manner if bile or pancreatic juice, or both, are kept from entering the intestine. He also found under such circumstances that the lacteals did not present the usual milky appearance which accompanies fat absorption.

The effect of a biliary fistula on fat absorption seems to be identical with that of a pancreatic fistula; exactly the same kinds of results have been recorded in the two cases. All observers are agreed that so much fat cannot be absorbed in presence of a biliary fistula as when bile has access to the intestine, but, while some find fat absorption practically arrested, others have observed that a considerable, nearly normal, amount of fat can still be disposed of. As in the case of absence of the pancreatic secretion, most of the unabsorbed fat is found in the fæces as fatty acid.[2]

Röhmann [3] also found that sodium soaps were not absorbed, but were converted into free fatty acids, and appeared as such in the fæces. Bidder and Schmidt [4] state that normal dogs can digest as much as seven times the quantity of fat which can be disposed of by dogs with fistula of the gall bladder, and that, while during fat absorption in a normal dog the lacteals are filled with milky chyle, they are, under similar conditions in a dog with a biliary fistula, filled with a yellow or slightly opalescent fluid.

C. Voit [5] estimates the average loss of fat at 22·2 to 34·7 per cent.; Munk,[6] at 33·1 per cent.; Röhmann,[7] at 48·5 to 58·4 per cent.; Noël Paton,[8] at 34·58 per cent.; Dastre,[9] at 57·65 per cent. Munk [10] found that the absorption of fats of high melting point (mutton) suffered more than that of fats of low melting point (hog's lard); of the former but 35·5 per cent. was utilised, of the latter 67 per cent. He also found that the free fatty acids in the absence of bile were absorbed equally well, in fact slightly better, than the corresponding neutral fats. Dastre [11] ligatured the *ductus choledochus*, and made a fistula between the gall bladder and small intestine much lower down (60–150 cm.); he observed, after a meal of fat, that the lacteals were only injected with milky chyle below the artificial point of entry of the bile. As Dastre himself remarks, the result is more elegant than decisive. It is only qualitative in character, and does not show quantitatively the share taken by pancreatic juice and bile in fat absorption. Hédon and Ville [12] established first a biliary fistula, and afterwards removed nearly all the pancreas, leaving just enough of the tail to preserve the animal alive, and destroying all communication with the intestine. In this manner both bile and pancreatic juice were kept out of the intestine, and under such conditions the digestion and absorption of fat was

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 171. Lewin removed the influence of both secretions by making a Thiry-Vella fistula of that part of the duodenum into which the ducts open.

[2] Röhmann, *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxix. S. 509; I. Munk, *Virchow's Archiv*, 1890, Bd. cxxii. S. 313; Hédon and Ville, *Compt. rend. Soc. de biol.*, Paris, 1892, tome xliv. p. 309. See, however, Dastre, *Arch. de physiol. norm. et path.*, Paris, 1891, tome xxiii. p. 186.

[3] *Loc. cit.*, S. 532. [4] "Die Verdauungssäfte," etc.

[5] "Beitr. z. Biologie," *Jubiläumsschrift f. v. Bischoff*, Stuttgart, 1882.

[6] *Virchow's Archiv*, 1890, Bd. cxxii. S. 302. [7] *Loc. cit.*

[8] *Rep. Lab. Roy. Coll. Phys.*, Edin., 1891, vol. iii. p. 214. The case was one of a complete biliary fistula in a woman.

[9] *Loc. cit.* [10] *Loc. cit.*, S. 324, 325. [11] *Loc. cit.* [12] *Loc. cit.*

studied. It was found that food passed very rapidly through the alimentary canal without much modification, scarcely any fat was absorbed, but it was nearly all converted into fatty acid.

These varied results may be summed up as showing that both the pancreatic juice and the bile are powerful aids in the digestion and absorption of fats, but neither is absolutely indispensable.

The view to be taken of the part played by bile and pancreatic juice in fat absorption must naturally vary with the view held as to the form in which fat is absorbed.

1. It may be urged that, in the absence of pancreatic juice, a sufficient supply of fatty acid is not set free for emulsification of the remainder. Since bile (or bile salts) very much hastens the fat-splitting action of pancreatic juice,[1] the absence of bile would have a very similar effect to that of pancreatic juice itself. A serious objection to this explanation lies in the fact that in defective absorption, due to the absence of either bile or pancreatic juice, nearly all the unabsorbed fat is found in the fæces as free fatty acid.

It might be claimed that this fat-splitting, probably by bacterial action, takes place much lower down in the intestine, at a less favourable position for absorption, and that a considerable part of the intestine is traversed before a sufficient amount of fatty acid is formed. But in the fæces as much as 80 per cent. of the total fat is as free fatty acid, while only about 5 per cent. is required for spontaneous emulsion; besides, the fat of the food contains nearly sufficient fatty acid to begin with, so that this contention has little weight.

2. Another view which has been held is, that in the absence of either bile or pancreatic juice the intestinal reaction is acid, so that no emulsion can take place, and hence the fat cannot be absorbed. It is not, however, claimed that such an acid reaction is due to free hydrochloric acid, since the remaining alkaline secretions are still more than sufficient to neutralise this, and active fat absorption has often been observed in presence of an acid reaction due to organic acids.

3. It has been supposed that the absence of the proteid of the pancreatic juice has an unfavourable effect on the formation of an emulsion (Minkowski).

4. A theory advanced by v. Wistinghausen[2] was that the bile aided fat absorption by mechanically wetting the epithelial cells with a fluid which rendered the passage of the fat easier. He claimed that oil stood higher in capillary tubes wetted with bile than in similar tubes wetted with water, and that oils or melted fats passed more rapidly through a membrane wetted with bile than through one wetted with water. These results have not, however, been confirmed by other observers.[3]

5. It has also been supposed that the bile directly stimulates (chemically) the epithelial cells of the intestine to increased fat absorption, and that in the absence of the bile this stimulus is absent. Under these conditions the epithelial cells either do not absorb fat as an emul-

[1] Rachford, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 87.

[2] Translation in *Arch. f. Anat. u. Physiol.*, Leipzig, 1873, S. 137, by J. Steiner. See also Schiff, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1857, Bd. ii. S. 345; Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. Supp. Heft, S. 91.

[3] Gröper, *Arch. f. Anat. u. Physiol.*, Leipzig, 1889, S. 505.

sion at all, or only absorb it at a greatly diminished rate.[1] There is no experimental evidence in support of this theory, and a great objection to it is that bile is constantly present in the intestine, and is not poured out in association with the presence of fat; such are not the proper conditions for a stimulus, which ought, if it is to be effective, to be intermittent, and only be called into action when required.

6. All the previous views rest on the assumption that the fats are absorbed in the form of an emulsion. If, on the other hand, the fats are absorbed in soluble forms as fatty acids or soaps and glycerin, the most obvious explanation of the action of bile and pancreatic juice in assisting absorption is, that these secretions increase the solubility of the fatty acids or soaps.

In the absence of bile or pancreatic juice, the fatty acids are not so soluble in the intestinal fluid, and so the absorption is defective, and the insoluble fatty acids appear in the fæces. In support of this the fact may be recalled that the bile salts possess the power of dissolving the insoluble soaps of the alkaline earths.[2]

Channels of absorption of the fats.—There is no doubt that the lacteals are the main channel by which the fats are carried away from the intestine, but it is by no means so clear that all the fat goes by this route. The amount of fat absorbed from the intestine after a fatty meal can easily be determined by weighing the amount of fat ingested, and that remaining in the alimentary canal when digestion is nearly complete, and taking the difference, which must be the amount absorbed. The amount of fat poured into the blood by the thoracic duct during the same period can also be determined, by inserting a cannula into the duct and collecting the chyle, from which the fat is afterwards extracted and then weighed. The amount thus carried by the thoracic duct during the period of active absorption is always much less than the total quantity absorbed; it has never been found to amount to more than 60 per cent., and is usually much less than this.[3] The fate of the balance of the fat is unknown; the first suggestion occurring to the mind, that it travels by the alternate path of the portal circulation, has not been found to fit the experimental facts. The portal vein during fat digestion does indeed contain an abnormal amount of finely emulsified fat, but so does all the blood of the body, and the presence of the fat is due to the admixture with the blood of the chyle carried by the thoracic duct. On diverting from the blood this supply of fat, by means of a cannula inserted into the thoracic duct, Zawilski found scarcely any fat in the blood during fat absorption. Neither is there any difference during fat absorption in the percentages of fat present in portal and carotid blood.[4] It would seem from this that almost all the fat is carried by the lacteals, but that part is removed somewhere in the lymphatic system between the lacteals and the opening of the thoracic duct; it may be in the lymphatic glands,[5] but the subject requires further investigation.

[1] Röhmann, *Arch. f. d. ges. Physiol.*, Bonn, Bd. xxix. S. 509; Minkowski, *Berl. med. Wchnschr.*, 1890, No. 15, S. 333; Lewin, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 186.

[2] See p. 392.

[3] Zawilski, *Arb. a. d. physiol. Anst. zu Leipzig*, 1867, Bd. xi. S. 147; Walther, *Arch. f. Anat. u. Physiol.*, Leipzig, 1890, S. 329; Frank, *ibid.*, 1892, S. 497; Munk u. Rosenstein, *Virchow's Archiv*, Bd. cxxiii. S. 484.

[4] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii., Supp. Heft, S. 95.

[5] See M. Foster, "Text-book of Physiology," pt. ii. p. 513.

After a full meal of fat, absorption, in the case of the dog, goes on for about thirty hours. At the height of absorption, the chyle in the thoracic duct may contain as much as 15 per cent. of fat. Twenty-one hours after a meal of 150 grms. of fat, Zawilski [1] still found in the stomach 9·74 grms., in the intestine 6·24 grms.; in thirty hours all but traces have disappeared from both stomach and intestine. Throughout this period of digestion, according to the same observer, the amount of fat in the intestine at any time remains practically constant (6·24 to 9·9 grms.), from which it would seem that the rate at which the fat is allowed to pass the pylorus is regulated by the amount of fat already present in the intestine.

None of the soap which may be formed during fat digestion and absorption probably ever enters the general circulation as such, but is reconverted into neutral fat beforehand; as Munk [2] has shown, soaps of the alkalies intravenously injected produce poisonous effects, closely resembling those obtained on injection of albumoses.

As might be expected from their similar chemical constitution, the lecithins are decomposed in the same manner as the fats by the steapsin of the pancreatic juice, the products of the reaction being glycerophosphoric acid, neurin, and fatty acids. These products are probably absorbed, as is shown by their absence in the fæces after the administration of lecithin by the mouth; as well as by the increase of phosphates in the urine after feeding on foods, such as yolk of egg, rich in lecithin.[3]

BACTERIAL DIGESTION.

The food in the alimentary canal is acted upon, not only by the digestive secretions and their enzymes, but to a greater or less extent by certain bacteria which are never entirely absent, although the amount of their action varies greatly, under healthy conditions, with the nature of the food and the class of animal. Under abnormal conditions the growth of these organisms may be greatly increased, and nutrition be seriously impaired, by their turning to their own uses the products of normal digestion, and leaving only for the service of the animal, degradation-products, inadequate or wholly unsuited for the purposes of its metabolism. Along with this increased growth of the bacteria normally present in the stomach, conditions may become so changed as to favour the growth of other bacteria, often pathogenic in character, which find under normal conditions no favourable soil for their growth in the intestinal contents, and thus various forms of disease may be introduced. We have here, however, only to deal with the changes induced by bacteria under a normal condition of the alimentary canal.

In dealing with the function of the free hydrochloric acid of the gastric juice, it has already been stated that this completely stops all bacterial action,[4] so that it is only in the first stage of gastric secretion, before the acidity has become marked, that any bacterial changes can occur.

Proteids are not attacked during this first short stage (twenty to forty minutes) of gastric digestion, but carbohydrates undergo to a

[1] *Loc. cit.* [2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1890, Supp. Bd., S. 116.

[3] A. Bokay, *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 157; see also Hasebroek, *ibid.*, 1888, Bd. xii. S. 148.

[4] See p. 364.

slight extent lactic fermentation.[1] A certain amount of decomposition of neutral fats also occurs during gastric digestion, yielding fatty acids, but it is not certainly known whether this is due to bacterial action or not.[2]

Intestinal bacterial digestion—Reaction of the intestine.—There is considerable difference of opinion both as to the amount of decomposition of foodstuffs due to bacterial action which goes on in the intestine, and as to the importance of such a decomposition as a normal factor in digestion.

The extent of bacterial action can evidently be more accurately gauged by the amount of bacterial decomposition products formed than by the presence in the intestine of bacteria, which may not there be in a very active condition.

Judged by this standard, the amount of proteid decomposition due to bacteria which takes place in the small intestine is excessively small, while a considerable amount takes place in the cæcum and large intestine generally. When carbohydrates and proteids are present in the same solution along with various bacteria capable of attacking them, the carbohydrates are first attacked, the action being accompanied by the formation of certain organic acids; at a later stage the proteids are attacked and decomposed. This has been shown by Maly,[3] who took mucous membrane of the stomach, placed it in a solution of cane-sugar, and kept the mixture at body temperature for several days. The lactic acid formed by the decomposition of the sugar was neutralised from time to time, and it was found that the process continued without a trace of putrefaction appearing, until all the sugar had been converted into lactate; then first appeared, often somewhat suddenly, an intense putrefactive odour, and the proteids began to be decomposed.

It is probable from this that, in the body, bacterial action on carbohydrates precedes that on proteids; and it has been supposed by some that such an action commences with considerable intensity in the duodenum, and persists throughout the entire length of the small intestine, so involving bacterial decomposition of a large share of the carbohydrate food.

This opinion rests chiefly on the observation that the acid reaction of the chyme in the stomach, due to hydrochloric acid, becomes replaced by an acid reaction, due to organic acids, in the small intestine. These organic acids are supposed to be set free by the action of certain bacteria, found in the small intestine, upon the carbohydrate food.

Such a result has been obtained by Macfadyen, Nencki, and Sieber,[4] from observations made on a case of *anus præternaturalis* in man, in which the fistula occurred quite at the lower end of the ileum. The intestinal contents arising from a mixed diet, consisting principally of animal food, had as they flowed from the fistula an acid reaction, equivalent to that of a solution of 1 per mille of acetic acid; this reaction was principally due to acetic acid,

[1] In this process hydrogen gas is set free along with lactic and traces of other acids; under abnormal conditions the amount of gas may be greatly increased. See E. Wissel, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xxi. S. 234, where the literature of this subject up to that date is given.

[2] Marcet, *Proc. Roy. Soc. London*, 1858, vol. ix. p. 306; Cash, *Arch. f. Anat. u. Physiol.*, Leipzig, 1880, S. 323.

[3] Hermann's "Handbuch," Bd. v. (2), S. 239.

[4] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 311, reprinted in *Journ. Anat. and Physiol.*, London, 1891, vol. xxv. p. 390. See also C. A. Ewald, *Virchow's Archiv*, 1879, Bd. lxxv. S. 409; and Jakowski, *Arch. d. sc. biol.*, St. Pétersbourg, 1892, tome i. p. 539.

accompanied by traces of fermentation and paralactic acid, volatile fatty acids, succinic acid, and bile acids. Hydrochloric acid was not present. The mixture had very little odour; occasionally the slight odour it had was faintly putrefactive, resembling indol, but usually it was more like that of volatile fatty acids. These authors state that it is the organic acids present in the small intestine which limit the bacterial decomposition of carbohydrates, and prevent the putrefaction of proteids.

On the other hand, Moore and Rockwood[1] state that the reaction of the intestine in various classes of animals (dog, cat, white rat, guinea-pig, and rabbit) is not normally acid throughout its entire length, and that the alkalinity increases in passing down the intestine.

The presence of fat in the food causes in carnivora an acid reaction, which persists until the lower third of the intestine is reached. This acid reaction is due to very weak organic acid, most probably to the acids of the fats dissolved by the agency of the bile.[2] The alkalinity is much greater in herbivora than in carnivora, although herbivora consume much more carbohydrate food than carnivora. Also, in carnivora, the alkalinity is markedly increased by carbohydrate food; this would not be the case if any considerable bacterial decomposition of carbohydrates took place in the small intestine, but the alkalinity would diminish from increased formation of organic acids. It is therefore probable that in these animals any extensive bacterial decomposition of carbohydrates that may occur, like that of proteids, takes place in the large intestine, and by analogy the same is probably the case in the human intestine.

Considerable importance has been attached to the normal action of bacteria in the intestine, and it has even been supposed that the presence of bacteria is essential to life. Such a view has recently been shown to be erroneous by an elaborate and painstaking research carried out by Nuttall and Thierfelder,[3] who obtained ripe fœtal guinea-pigs, by means of a Cæsarean section, carried out under strict antiseptic precautions. They introduced the animals immediately into an aseptic chamber, through which a current of filtered air was aspirated, and fed them hourly on sterilised milk day and night for over eight days.

The animals lived and throve, and increased as much in weight as healthy normal animals, subjected to a similar diet for the purpose of controlling the results. Microscopic examination at the end of the experiment showed that the alimentary canal contained no bacteria of any kind, nor could cultures of any kind be obtained from it. The same authors, in a subsequent paper, describe the extension of their research to vegetable food; this was also digested in the absence of bacteria. Under such conditions cellulose was not attacked; hence they consider that the chief function of this material is to give bulk and a proper consistency to the food, so as to suit the conditions of herbivorous digestion.

Action of the intestinal bacteria on proteids.—The changes brought about in the intestine are very similar and probably identical with those which occur when proteids undergo putrefaction in the air, with this important exception, that those putrefactive bacteria which produce the class of poisonous nitrogenous (alkaloidal) bases known as ptomaines do not grow under normal conditions in the intestine. This may be due to the intestinal contents not furnishing a suitable medium for their growth, or to the time of putrefaction in the intestine not being sufficiently prolonged. Ptomaines, and especially poisonous ones, are formed only in

[1] *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 373.
[2] See "Digestion and Absorption of Fats," p. 454.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xxi. S. 109; 1896, Bd. xxii. S. 62.

the later stages of putrefaction. It has also been suggested that the cause may be the absence of oxygen from the intestine, the ptomaine-forming bacteria being aërobic. That the bacteria which produce ptomaines are present in the intestine, is shown by the fact that cultures producing ptomaines may be obtained by sowing from the intestinal contents into suitable media.[1] At any rate, ptomaines even in traces are not to be found in the intestinal contents; and it is fairly certain that they are not produced there, as most of them, absorbed even in minute doses, are capable of producing profound toxic effects.

The first stages in the action of bacteria on proteids are very similar to those induced by trypsin; they can also be brought about by enzymes extracted from the bacteria,[2] but, according to Kühne,[3] are not due to trypsin, which is never formed in bacterial putrefaction.

The first action is the solution of the proteid, if this is not already dissolved. Solution takes place much more slowly than in the case of the digestive enzymes, the complete solution of fresh fibrin thoroughly infected with intestinal bacteria in faintly alkaline solution being a process of some days' duration.[4] Albumoses, peptones, and the other products of tryptic digestion are next formed, but the amount of these present at any time is never great, since they are gradually broken up as they are formed into more advanced degradation products. In the presence of albumoses or peptones, the native proteids are very faintly attacked by the bacteria, until these have first been disposed of. Neumeister[5] has shown that, when peptone is added to putrefying blood or proteid, its quantity is not increased by the continuance of the putrefactive process, but rather diminished until it finally disappears.

The proteid molecule probably contains a large number of both fatty and aromatic radicles, but all those belonging to the aromatic group yield, *under the action of trypsin*, only one substance, namely, tyrosine. The same is true of all artificial modes of decomposition which do not act too intensely on the primary products of decomposition.[6] But with the decomposition produced by bacteria, the case is different, and several aromatic compounds are formed.

These are in part produced by further action on tyrosine, formed in an earlier stage, and in part spring from a specific action of the bacteria on the proteid, without the intervention of tyrosine. Only some of these products of bacterial decomposition have been hitherto found in the intestinal canal; the others are either, under the different conditions, not formed there, or are so rapidly absorbed and altered that they cannot be detected in the intestinal contents.

The chief aromatic compounds derived from the bacterial decomposition of proteids are:—(*a*) Tyrosine, and its derivatives, paraoxyphenylpropionic acid (hydroparacumaric acid), and paraoxyphenylacetic acid, as well as phenylpropionic and phenylacetic acids,[7] of which the foregoing are the oxy- or hydroxy-acids, also parakresol and phenol.

[1] Brieger, *Deutsche med. Wchnschr.*, Leipzig, 1887, S. 469; Baumann u. Udransky, *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 579.

[2] See p. 313.

[3] *Untersuch. a. d. physiol. Inst. zu Heidelberg*, 1878, Bd. i. S. 291.

[4] Bienstock, *Ztschr. f. klin. Med.*, Berlin, 1884, Bd. viii. S. 1.

[5] *Ztschr. f. Biol.*, München, 1890, Bd. xxvii. S. 335; "Lehrbuch," Th. 1, S. 207.

[6] Kühne obtained indol by the fusion of proteid with caustic alkali, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1875, Bd. viii. S. 206.

[7] See E. Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 491.

(*b*) Substances formed directly and not from tyrosine—indol, skatol, and skatolcarbonic acid.

Of these substances, Zumft[1] found indol, skatol, phenol, and parakresol in the large intestine of man, but skatolcarbonic acid was absent; this latter acid has not yet been detected in the intestine. According to E. Salkowski, it is excreted unchanged in the urine, and he states that he has detected it in normal urine.[2]

These several substances may now be considered *seriatim*.

Derivatives of tyrosine formed in putrefaction.[3]—*Hydroparacumaric acid*, or *paraoxyphenylpropionic acid* ($HO.C_6H_4.C_2H_4.COOH$) crystallises from water in anhydrous monoclinic crystals, melting at 125°–128° C., soluble in water, alcohol, and ether. It gives a transient blue coloration with ferric chloride, and a red coloration or red precipitate when boiled with Millon's reagent. It is the oxy-acid of phenylpropionic acid, which has also been found among the putrefaction-products of proteids. Phenylpropionic acid crystallises in slender needles, melting at 47°–48° C. (B-Pt. 280° C.). As follows from its constitution, its solutions do not give Millon's reaction.

Paraoxyphenylacetic acid ($HO.C_6H_4.CH_2.COOH$) crystallises from water in prismatic crystals, melting at 148° C., and soluble in water, alcohol, and ether. With ferric chloride it gives a faint violet coloration, changing to a dirty grey-green. It also gives Millon's reaction. Phenylacetic acid crystallises in scales, which melt at 76°·5 C.

Phenol and *parakresol*[4] are also formed in the bacterial decomposition of tyrosine; they are absorbed from the alimentary canal, and after conversion into ethereal sulphates are excreted in the urine. The amount of these ethereal sulphates in the urine gives a measure of the amount of bacterial decomposition going on in the intestine.[5]

Tyrosine and its derivatives are very closely related to one another. In the derivation of these compounds, according to Baumann,[6] tyrosine (paraoxyphenyl-α-amidopropionic acid) undergoes reduction, ammonia being split off, and hydroparacumaric acid (paraoxyphenylpropionic acid) formed. This compound, by a series of oxidations, accompanied by a splitting off of carbon-dioxide, yields paraoxyphenylacetic acid and parakresol. Parakresol is said to similarly yield phenol.

These changes are illustrated by the following equations :—

$$C_6H_4\begin{cases}OH\ (p)\\ CH_2.CH(NH_2).COOH\end{cases} + H_2 = C_6H_4\begin{cases}OH\ (p)\\ CH_2.CH_2.COOH\end{cases} + NH_3$$

(paraoxyphenyl-α-amidopropionic acid or tyrosine) — (paraoxyphenylpropionic acid or hydroparacumaric acid)

$$2C_6H_4\begin{cases}OH\ (p)\\ CH_2.CH_2.COOH\end{cases} + 3O_2 = 2C_6H_4\begin{cases}OH\ (p)\\ CH_2.COOH\end{cases} + 2CO_2 + 2H_2O$$

(paraoxyphenylpropionic acid) — (paraoxyphenylacetic acid)

[1] *Arch. d. sc. biol.*, St. Pétersbourg, 1892, vol. i. p. 497.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 32.

[3] See Baumann. *Ztschr. f. physiol. Chem.*, Strassburg, 1877–1880, Bd. i. S. 60; iv. S. 304; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1879, Bd. xii. S. 1450; 1880, Bd. xiii. S. 279; Baumann and Brieger, *Ztschr. f. physiol. Chem.*, Strassburg, 1879, Bd. iii. S. 149; E. and H. Salkowski, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1879, Bd. xii. S. 648; E. Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1878–9, Bd. ii. S. 420; Weyl, *ibid.*, 1877–9, Bd. i. S. 339; iii. S. 312.

[4] For a description of the physical and chemical properties of these bodies, see Gamgee, "Physiological Chemistry of the Animal Body," 1893, vol. ii. p. 434.

[5] Baumann, *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 123.

[6] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1879, Bd. xii. S. 1450.

$$C_6H_4\begin{cases}OH\ (p)\\CH_2.COOH\end{cases} = C_6H_4\begin{cases}OH\ (p)\\CH_3\end{cases} + CO_2$$

(paraoxyphenylacetic acid) (parakresol)

$$2C_6H_4\begin{cases}OH\ (p)\\CH_3\end{cases} + 3O_2 = 2C_6H_4\begin{cases}OH\\H\end{cases} + 2CO_2 + 2H_2O$$

(parakresol) (phenol)

By a process of reduction, the oxy-acids probably yield the phenylpropionic and phenylacetic acids which have been found, thus :—

$$C_6H_4\begin{cases}OH\ (p)\\CH_2.COOH\end{cases} + 2H = C_6H_5.CH_2.COOH + H_2O$$

(paraoxyphenylacetic acid) (phenylacetic acid)

Aromatic bodies found in putrefaction not formed from tyrosine.—Indol, skatol, and skatolcarbonic acid are not formed by bacterial action on tyrosine, and their mode of formation is not very clearly known. According to Baumann,[1] they are not primary products of bacterial action on the proteid molecule, but are formed in the decomposition of an intermediate body, which is soluble in a mixture of alcohol and ether. E. and H. Salkowski [2] support this conclusion. Nothing further is known of this intermediate substance, except that it is not peptone. Neumeister [3] considers it possible that these substances may be synthetically built up by the bacteria from simpler aromatic compounds. Indol and skatol are formed from this mother substance in varying proportion, probably due to the action of different bacteria, but these have never been isolated.

Indol, skatol, and skatolcarbonic acid belong to the indigo group of aromatic compounds. Indol on oxidation yields indoxyl, and on further oxidation, this yields indigo blue. By an inverse process of reduction from indigo blue, indol can be obtained.[4] To indol and skatol the fæces owe to a great extent their peculiar unpleasant odour.

Indol,[5] $C_6H_4\begin{cases}NH\\CH\end{cases}CH$, crystallises from water in small scales (M. P., 52° C., B. P., 245° – 246° C.). It is fairly soluble in hot, less so in cold water, and is easily soluble in alcohol, ether, chloroform, benzol, and petroleum ether. It distils over with steam; this property may be used to separate it from other putrefaction products.[6] In long-continued putrefaction, indol gradually disappears; according to Salkowski, this is due to evaporation.

Indol may be recognised by the following tests :—

1. A wooden match moistened with strong hydrochloric acid and then dipped into an alcoholic solution of indol turns a cherry-red colour.

[1] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1880, Bd. xiii. S. 284.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1884, Bd. viii. S. 454 ; see also Nencki and Bovet, *Monatsh. f. Chem.*, Wien, 1889, Bd. x. S. 506.

[3] "Lehrbuch. d. physiol. Chem.," Jena, 1893, Th. 1, S. 209.

[4] Nencki, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1875, Bd. viii. S. 722 ; Baumann, u. Brieger, *Ztschr. f. physiol. Chem.*, Strassburg, 1879, Bd. iii. S. 254.

[5] Baeyer, *Ann. d. Chem.*, Leipzig, Bd. cxl. S. 295 ; Supp. Bd. vii. S. 56.

[6] For method of isolation from these, see Gamgee, "Physiological Chemistry, etc.," vol. ii. p. 421 ; or E. and H. Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1884, Bd. viii. S. 417.

2. An aqueous solution of indol treated with *fuming* nitric acid turns a bright red colour, and on standing a red precipitate is formed.

3. When sodium nitroprusside is added to a very dilute solution of indol, and afterwards caustic soda, the mixture turns a deep violet-blue, passing into a pure blue on making faintly acid with acetic acid, and disappearing with excess of acid (Legal's reaction).[1]

Skatol,[2] $C_6H_4\begin{<}{NH}{C.CH_3}\!\!>\!\!CH$, is methyl-indol; it crystallises in similar form to indol (M. P., 95° C., B. P., 265° – 266° C.). It also possesses much the same solubilities as indol, and is volatile with steam. Passed through a red-hot tube, it decomposes and yields indol.

It is distinguished, in addition to its physical properties, by the following tests:—

1. Instead of a red precipitate, as in the case of indol, it gives a milky turbidity when treated with fuming nitric acid.

2. In Legal's test (*vide supra*) it gives an intense yellow, turning violet with acid.

3. It dissolves in concentrated hydrochloric acid, giving a highly coloured solution.

Both indol and skatol, dissolved in benzol in concentrated solution, give, with a saturated solution of picric acid in benzol, a crop of fine red crystals. When the compound of indol and picric acid is treated with caustic soda, and distilled, the indol is decomposed; under similar conditions the skatol picric acid compound yields skatol which is not decomposed.

Skatol carbonic acid,[3] $C_6H_4\begin{<}{NH}{C.CH_3}\!\!>\!\!C\cdot COOH$, crystallises in scales (M. P., 164° C.), sparingly soluble in water, easily soluble in alcohol and ether. Heated above its melting point, it breaks up into skatol and carbon-dioxide.

It may be identified by the following tests:—

1. Its aqueous solution, treated with pure nitric acid and afterwards with potassium nitrite solution, turns a cherry-red colour, and deposits a red precipitate, which is dissolved by acetic ether.

2. Its aqueous solution, treated with an equal volume of hydrochloric acid (sp. gr. 1·2), and afterwards with dilute bleaching powder solution, gradually turns a purple-red colour, and, after long standing, deposits a purple-red precipitate, easily soluble in alcohol.

3. A very dilute solution (1 in 10,000 of water), treated with a few drops of hydrochloric acid, then with a few drops of a very dilute solution of ferric chloride, and heated, gives an intense violet colour. More concentrated solution gives an intense cherry-red colour.

The aromatic compounds resulting from bacterial decomposition in the intestine are to a considerable extent absorbed. Tyrosine absorbed as such disappears; it is decomposed and completely oxidised in the tissues without the formation of urea. The non-nitrogenous substances resulting from its decomposition by bacteria (as well as indol and skatol) are not completely oxidised, but are excreted in modified form in the urine, combined chiefly with sulphuric acid, as ethereal sulphates, but also in part with glycocoll and glycuronic acid. In this way the poisonous properties of the phenols and similar compounds are removed, for the ethereal sulphates formed are very

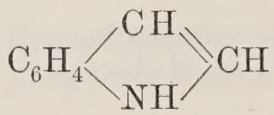
[1] *Breslau. ärztl. Ztschr.*, 1893.

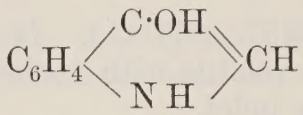
[2] Brieger, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1877, Bd. x. S. 1028.

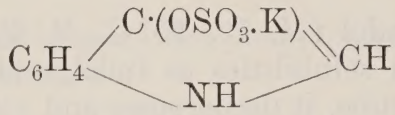
[3] E. and H. Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 8.

stable compounds, which are excreted unchanged; thus phenol and kresol are eliminated as potassium salts of phenylsulphuric acid and kresolsulphuric acid respectively, $SO_2\langle^{OC_6H_5}_{OK}$, $SO_2\langle^{OC_6H_4—CH_3}_{OK}$.

Indol and skatol are to a considerable extent excreted with the fæces. The portion which is absorbed is first oxidised, yielding indoxyl and skatoxyl, and these are then united to form sulphates with potassium-hydrogen sulphate, thus :—


(indol)


(indoxyl)


(potassium indoxylsulphuric acid)

The aromatic oxy-acids in part are found in the urine as simple salts, and in part combined with sulphuric acid. The simple aromatic acids (phenylacetic and phenylpropionic acids) are chiefly found united with glycocoll. The phenylpropionic acid is first changed into benzoic acid, and then unites with glycocoll to form hippuric acid (benzoylglycocoll, $C_6H_5.CONH.CH_2.COOH$). The phenylacetic acid unites directly with glycocoll to form phenaceturic acid ($C_6H_5.CH_2.CO—NH.CH_2.COOH$).

Besides these substances belonging to the aromatic series, there are formed, during the putrefactive decomposition of proteids, a number of substances belonging to the fatty series. The chief of these are leucine, the ammonium salts of a number of volatile fatty acids (caproic, valerianic, and butyric), methane, hydrogen, sulphuretted hydrogen, and methylmercaptan ($CH_3.SH$).

Action of the intestinal bacteria on carbohydrates.—The carbohydrates suffer much more bacterial decomposition in the intestine than do the proteids. Not only are the sugars formed in digestion attacked, but starch is directly attacked by some bacteria,[1] and cellulose, so far as it is decomposed, owes its changes to bacterial action. The products formed in such bacterial actions on the carbohydrates are simpler in their composition than those produced during putrefaction; they consist chiefly of ethyl alcohol, lactic (active and inactive), butyric, and succinic acids, accompanied by carbon-dioxide and hydrogen.

Nencki, Macfadyen, and Sieber[2] isolated seven different intestinal bacilli, of which five acted only on carbohydrates (dextrose), and the other two mainly on proteids.

Cellulose is altogether unattacked by any of the digestive juices *in vitro*; nevertheless it disappears to a very considerable extent in natural digestion. Experiments on herbivora show that 60 to 70 per cent. of the cellulose disappears,[3] and even shavings and paper mixed with hay and given to sheep only partially reappear in the fæces. Experiments on man show that, according to the condition and form of the cellulose, amounts varying from 4 to 60 per cent. are digested.[4]

Of the manner in which this cellulose is broken up or dissolved we know nothing with certainty. Bunge[5] supposes that the epithelial cells

[1] Wortman, *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 293; Lauder Brunton and Macfadyen, *Proc. Roy. Soc. London*, 1889, vol. xlvi. p. 542.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 311. Reprinted in *Journ. Anat. and Physiol.*, London, 1891, vol. xxv. p. 390.

[3] Haubner, *Ztschr. f. Landwirthschaft*, 1885, S. 177.

[4] Weiske, *Ztschr. f. Biol.*, München, 1870, Bd. vi. S. 456; v. Knieriem, *ibid.*, 1885, Bd. xxi. S. 67.

[5] "Lehrbuch der physiol. u. path. Chem.," 1894, S. 174.

of the intestine possess the function, by means of a ferment, of dissolving the cellulose; this may be so, but no such ferment has ever been shown to exist. Bunge supports his suggestion by analogy with the action of some unicellular organisms on cellulose.[1]

Exposed to the action of certain organisms, cellulose undergoes fermentation with the setting free of marsh gas (CH_4), and the formation of acetic and butyric acids; how much of the altered cellulose goes in this way in the digestive process is unknown. Tappeiner[2] tested the action of the intestinal bacilli on cotton-wool, by soaking this in a 1 per cent. solution of bouillon and inoculating with the bacilli. Fermentation with development of gas commenced, and there were formed in the solution free fatty acids (up to and including valerianic acid), while the cotton-wool nearly all dissolved. The gases set free were marsh gas and carbon-dioxide. The nature of the products varies with the organism acting on the cellulose; thus Hoppe-Seyler[3] obtained the same gases accompanied by a dextrin-like substance, by the action of pond bacteria on cellulose in the form of filter paper, but did not observe the formation of any fatty acids.

Experiments on the artificial digestion of cellulose in the form of new hay were made by Hofmeister,[4] who showed that the intestinal juices of the horse were capable of dissolving nearly 80 per cent. of this material. No formation of sugar but some fermentation and development of gas were observed.

The most important uses of cellulose lie, however, not in its value as a nutrient foodstuff, but in giving bulk and looseness to the food and in mechanically inducing peristalsis by irritation of the intestine.[5] For this reason cellulose becomes an absolute necessity for animals with a long intestine, such as the herbivora. Rabbits fed on food free from cellulose rapidly die from intestinal inflammation; but if the same food be mixed with such an inert substance as horn shavings, nutrition goes on quite normally, and the animals continue in perfect health, although the horn shavings remain entirely unaltered.[6] The carnivora with their short intestine require no such aid to peristalsis; but in animals in an intermediate position, such as man, bulky or cellulose-containing food, while not indispensable, is from a dietetic point of view exceedingly desirable.

Action of the intestinal bacteria on fats.—Under a normal condition of the intestine, it is probable that very little decomposition of the fats by bacteria takes place, but under abnormal conditions, such as the absence of the bile or pancreatic juice, they are almost completely decomposed into fatty acids, which pass out unabsorbed along with the fæces. The first action of bacteria on fats consists in setting free the corresponding fatty acids; these are afterwards partially broken down into mixtures of fatty acids lower in the series.[7]

Lecithins undergo a similar decomposition by bacteria under anærobic conditions; they at first are split up into glycerophosphoric acid, fatty acids, and choline. Afterwards, the choline is decomposed with

[1] *E.g.*, Vampyrella; Cienkowski, *Arch. f. mikr. Anat.*, Bonn, 1865, Bd. i. S. 203.
[2] *Ztschr. f. Biol.*, München, 1884, Bd. xx. S. 52; *ibid.*, 1888, Bd. xxiv. S. 105.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 401.
[4] *Arch. f. wissensch. u. prakt. Thierh.*, Berlin, 1885, Bd. xi. S. 46.
[5] Bunge, "Physiological and Pathological Chemistry," 1894, p. 75.
[6] v. Knieriem, *Ztschr. f. Biol.*, München, 1885, Bd. xxi. S. 67.
[7] Gröger, *Ztschr. f. ang. Chem.*, Berlin, 1889, S. 62.

formation of carbon-dioxide, methane, and ammonia; but if air is present, neurin and muscarin are also formed in the process.[1]

COMPOSITION OF FÆCES.

Amount and consistency.—The consistency of the contents of the small intestine in the upper two-thirds to three-fourths of its length is fairly uniform, the amount of water absorbed in this part being approximately balanced by that added in the digestive fluids. But in the lower part of the small intestine the amount of water absorbed begins to exceed that secreted; the intestinal contents become thicker, and the thin fluid, with lumps of solid, undigested, or partially digested food of various kinds floating in it, which is usually found in the higher part of the intestine, is replaced by a pasty or semi-solid mass. As this mass passes along the large intestine the process of absorption continues with increased intensity, and a large amount of water, together with anything it holds in solution of service to the economy, is removed. The residue, a complex mixture of various useless or unused material, usually acquires the consistency of a soft solid before the completion of the process, and is finally ejected from the rectum. The consistency of the fæces, as well as the amount excreted per diem, varies within wide limits, with the character of the food and the duration of its passage through the intestine. Even in the rectum the process of absorption goes on, and fæces retained here become dry and hard. The fæces passed on a vegetable diet, or on a diet containing a liberal allowance of vegetables, are both much softer (*i.e.* contain more water) and much greater in total quantity of dry solids than those on a meat diet alone. The increase in the quantity of solids is due to the vegetable food containing a much higher percentage of undigestible tissue. The softer consistency arises from the stimulation of the mucous membrane by the undigested remnants of the vegetable tissue, causing increased peristalsis, so hastening the transit through the intestine, and shortening the period of absorption. This stimulating action of vegetable food adds greatly to its value in a mixed diet. In consequence of the absence of this stimulus, the period of defæcation is greatly prolonged on a purely flesh diet, and may only take place at intervals of several days. The amount of fæces daily excreted by man on a mixed diet averages, according to Voit,[2] 120 to 150 grms., containing 30–37 grms. of dried solids; on a vegetarian diet, the average amount obtained was 333 grms., containing 75 grms. of dry material.

Colour.—The colour of the fæces varies greatly, being mainly influenced by the nature of the food. On a diet of meat, the colour is dark brown to pitch black, due to hæmatin and to ferrous sulphide, formed by the action on hæmoglobin-derivatives, of sulphuretted hydrogen generated by bacteria in the intestine. Administration of iron or bismuth salts produces a similar effect. A liberal allowance of bread, especially of the coarser varieties, in the food, gives rise to light yellow-coloured fæces. Fat, when eaten in greater quantity than the animal requires, is excreted with the fæces chiefly as fatty acids and soaps, and

[1] Hasebröck, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 148.
[2] *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 264.

causes the fæces to have a yellowish or clay-coloured appearance. Such fatty stools also result when imperfect fat absorption is caused by stoppage of the bile duct. The derivatives of bile pigments also contribute to the colour of the fæces,[1] and part of the brown colour of normal fæces arises from these, although it is probably due in greater measure to hæmatin. Administration of calomel, by arresting bacterial decomposition, prevents the reduction of the bile pigment, which then appears in the fæces as biliverdin, and produces a green colour. The similar colour of meconium shows that bacteria are absent in the fœtal intestine. Green-coloured fæces are also excreted for some time after birth, until the normal bacteria of the intestine gradually acquire possession, when the biliverdin is reduced and the fæces assume a brown colour.

Reaction.—The reaction of the fæces is also variable. According to Hammarsten,[2] they may often be alkaline on the surface, from contact with the intestinal mucous membrane, while acid within the mass. Gamgee[3] states that the fæces in man are normally alkaline, and very exceptionally present an acid reaction. Wegscheider[4] found the fæces normally acid in infants.

Composition.—The fæces are an exceedingly complex mixture, containing substances of various origin and constitution, soluble and insoluble, derived from the food, the bile, and the detritus of the intestinal surface.[5] The number of these components is so large, and the amounts in which they are present so variable, that tables of quantitative composition possess little value.

The undissolved substances consist of fragments of undigested food, such as pieces of vegetables, muscle fibres, connective tissue, elastic fibres, and small masses of casein and fat. The amount of these is largely increased when the supply of food taken in is more than sufficient to satisfy the demands of the body. A microscopic examination further shows epithelial cells derived from the intestine, starch granules, fat globules, and occasionally crystals of magnesium and calcium phosphates, and of ammonia-magnesium phosphate. Besides these, there is present the indigestible residue of various foodstuffs, such as nucleins from nucleo-proteids, keratin from epidermal structures, and hæmatin from hæmoglobin.

The mineral salts present vary with the food, but consist chiefly of the phosphates of the alkaline earths, with small quantities of silica and phosphate of iron.

The other constituents include mucin, derived from the various secretions, mainly from the mucous membrane of the intestine; indol, skatol, volatile fatty acids, ammonia, sulphuretted hydrogen, and methane,

[1] There is some difference of opinion on this point. Gamgee ("Physiological Chemistry of the Animal Body," vol. ii. p. 458) states that the brown colour of normal fæces is due to hydrobilirubin; Hammarsten ("Lehrbuch der physiol. Chemie," Aufl. 3, S. 283), that the decomposition products of the bile pigments have little influence on the normal colour of the fæces.

[2] "Lehrbuch der physiol. Chemie," Aufl. 3, S. 284.

[3] "Physiological Chemistry of the Animal Body," vol. ii. p. 457.

[4] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, Bd. vi. S. 482.

[5] The mucous membrane of the intestine and its secretion furnish a considerable quota to the fæces; this is shown by an experiment due to Hermann (*Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 93), who separated a loop of intestine, cleared it of contents, sewed its two ends together so that it formed a ring, restored the continuity of the remainder of the intestine, and closed the wound. In a few days it was found, on killing the animal, that this loop was filled with a mass resembling fæces in appearance.

which are formed in bacterial decomposition;[1] and the products yielded by the bile, stercobilin, cholesterin, and *traces* of bile acids.[2]

Stercobilin[3] is a reduction compound of the bile pigments formed in their passage along the intestine, and is probably identical with urobilin and hydrobilirubin. Normally, all the bile pigment is reduced to this substance, but bilirubin has been observed in the fæces under pathological conditions.

Excretine is a crystalline body, described by Marcet[4] as occurring only in human fæces. It is very soluble in boiling alcohol, and may be mechanically thrown down from an alcoholic extract of the fæces by adding milk of lime. The precipitate is washed with water, dried and extracted with a mixture of alcohol and ether. On concentration of the solution by evaporation, impure excretine crystallises out. This is dissolved in hot alcohol, decolorised by animal charcoal, and obtained pure on recrystallisation in acicular four-sided prisms, melting at 92°–96° C. It is insoluble in water, hot or cold; sparingly soluble in cold, readily in hot alcohol; very soluble in ether; and the solutions are neutral in reaction. Its quantitative composition gives the formula $C_{78}H_{156}SO_2$. Hinterberger[5] states that, by repeated crystallisation, it may be obtained free from sulphur, and then has the empirical formula $C_{20}H_{36}O$; with bromine this yields a substitution compound, $C_{20}H_{34}Br_2O$.

Excretoleic acid is a body described by Marcet,[6] who obtained it on cooling a hot alcoholic solution of human fæces. On exhausting with ether, a green ethereal solution is obtained, which, on evaporation, leaves an oily residue of a dark green colour and acid reaction, melting at 25°–26° C.

Meconium is the name given to the contents of the large intestine in the fœtus, which are expelled at, or after, birth. It is a dark brownish-green mass of acid reaction. It contains 20 to 30 per cent. of dried solids, which consist of mucin, bile pigments (biliverdin and bilirubin), bile acids, cholesterin, fats, fatty acids, calcium and magnesium phosphates and sulphates.

In addition it contains a substance giving two absorption bands, one to the red side of the D line, and the other, broader and darker, between the D and E lines.

[1] The odour of the fæces arises from these bodies. [2] See p. 391.

[3] Vanlair and Masius, *Centralbl. f. d. med. Wissensch.*, Berlin, 1871, No. 24, S. 369; see also under "Bile," p. 388.

[4] *Med. Times and Gaz.*, London, 1858, N. S., vol. xvii. p. 156.

[5] *Ann. d. Chem.*, Leipzig, 1873, Bd. clxvi. S. 213. [6] *Loc. cit.*

THE SALIVARY GLANDS.[1]

By J. N. Langley.

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Some Anatomical Characters of the Salivary Glands.

In the dog and cat, the sublingual gland enlarges at its end, and loses its flattened form ; the enlarged end is closely attached to the submaxillary gland, and is enclosed in the firm capsule of this gland, so that at first sight it appears to form part of it.

The ducts from the lobules of the submaxillary gland unite, either in the connective tissue which stretches from the hilus of the gland, or in the hilus itself. The gland duct—the duct of Wharton—runs from the hilus to its opening underneath the tongue, without receiving, except in rare cases, any further accession.

The sublingual gland in about its anterior two-thirds consists of flattened lobules, the ducts of which enter the main duct on its course

[1] Physiological investigations on the salivary glands have, for the most part, been carried out on the larger glands, namely, the submaxillary, the parotid, and the sublingual. But such conclusions as we may be able to form with regard to these, we may apply with little change to the numerous smaller glands which pour their secretion into the mouth and pharynx, and, indeed, to the lachrymal glands and glands of the nasal mucous membrane also. Both in histological and physiological characters the lachrymal gland resembles an albuminous salivary gland. It receives cranial secretory fibres by way of the lachrymal branch of the fifth nerve ; the origin of these fibres from the medulla has not been investigated. It receives sympathetic fibres by way of the cervical sympathetic and the blood vessels of the gland. Secretion can be produced reflexly by stimulating most, if not all, sensory nerves.

The animals on which investigations have been made are chiefly the dog, cat, and rabbit, the horse, ox, and sheep.

past them. Thus the gland has no proper hilus. The duct runs parallel to and a little laterally of Wharton's duct.

The main blood supply, both to the submaxillary and the sublingual gland, is derived from a branch given off by the external maxillary artery. The submaxillary division of this branch runs to the hilus of the gland, and there divides. The submaxillary gland receives also one or two small branches from the great (or posterior) auricular artery, where this curls round the digastric muscle.

The veins of the submaxillary gland are variable in position, and somewhat variable in number. There are generally two; they run a short course, about half a centimetre, and then one enters the internal, and the other the external, maxillary vein, a little before these unite to form the external jugular vein.

In the dog and cat there is a fairly large gland situated in the orbit, and hence called the orbital gland. Its duct opens near the second upper molar tooth. The orbital gland corresponds to the large buccal gland, which in some animals is called the superior molar.

In the rabbit, the only point we need mention is that the parotid gland consists of two larger and thicker portions, a medial and a lateral, connected by a thin central portion. By appropriate arrangement, the thin central portion can be observed under a microscope, and the appearance of the gland cells in life, and the variations of the blood flow in varying conditions, can be observed.

Occasionally in the dog, and more constantly in some other animals, for instance the guinea-pig, a small gland, called by Klein [1] the inferior admaxillary, pours its secretion into the duct of the parotid. It may be regarded as a separated lobule of the parotid gland, although its secretory cells are mucous, instead of being albuminous, thus differing from the parotid secretory cells in general (cf. below, p. 478).

In a considerable number of animals, a small mucous gland is attached to the outer anterior end of the submaxillary gland. This was described by Klein in the guinea-pig, and called by him the superior admaxillary. The duct of this gland, according to Ranvier, runs parallel to and on the outer side of Wharton's duct, but does not join it. He calls it the retrolingual gland.

Ranvier [2] considers that the customary use of the term sublingual gland is in many cases erroneous. The sublingual, he defines as a gland which has a number of separate ducts—the ducts of Rivini. But, besides the sublingual, another gland occurs, which he calls the retrolingual gland. This is characterised by having a single duct—the duct of Bartholin—running parallel to Wharton's duct. An animal may have both sublingual and retrolingual, as the guinea-pig, rat, and hedgehog; or the retrolingual may be absent, as in man, horse, sheep, and rabbit; or, again, the sublingual may be absent, as in the dog and cat. Thus Ranvier considers the gland usually called the sublingual in the dog and cat to be the retrolingual.

In different classes of mammals, the relative development of the salivary glands varies. Thus in the horse the parotid is four to five times the weight of the submaxillary gland, in the sheep and ox the weights are not very different, in the dog the submaxillary gland is slightly heavier than the parotid.

[1] *Quart. Journ. Micr. Sc.*, London, 1881, p. 114.
[2] "Étude anatomique des glands," *Arch. de physiol. norm. et path.*, Paris, 1886.

The following table is taken from Colin:[1]—

Weight in Grammes of →	Parotid.	Submaxillary.	Sublingual.
Horse	400	86	23
Ox	283	298	43
Sheep	43	36	4

Some Histological Characters of the Salivary Glands.

It would be outside the scope of this account to give a detailed description of the ducts, ductules, terminal tubes, lymphatics, and other histological features of the several glands. But some of the histological features have so intimate a relation to physiological observations, that it is not advisable to pass them by without notice.

The narrow ductule, proceeding from a duct, commonly divides, and each secondary ductule widens more or less suddenly into a tube of secreting cells; each tube gives off curved branches, and these also may give off similar branches; thus, a clump of tubes is formed around the primary ductule. The terminal tubes are usually called alveoli, and their cells alveolar cells.

The alveolar cells may be classified according to the chemical nature of the substances they secrete. A step in this direction was taken by Heidenhain, who divided the cells into mucous cells which secrete mucin, and albuminous cells which secrete some form of proteid. There is good evidence that the typical albuminous cell does not secrete any mucin, and there is some evidence that the typical mucous cell does not secrete any proteid, and on this basis it is apt to be assumed that all the alveolar cells secrete either mucin only, or proteid only. It should, however, be remembered that this is an assumption; it is possible that some alveolar cells secrete both mucin and proteid.

In any one salivary gland all the alveolar cells may be mucous or all may be albuminous, or some of them may be mucous and some albuminous. Further, in different glands, the relative number of the two kinds of cells varies in nearly all possible proportions. The nomenclature in use takes notice of the broad distinctions only. Glands which consist almost entirely of albuminous cells are called albuminous glands, those which consist chiefly of mucous cells are called mucous glands. The glands of intermediate structure are commonly placed in the class of mucous glands, unless the proportion of mucous to albuminous cells is very small.

The term "mixed gland" was introduced for certain special cases; for example, the submaxillary gland of the guinea-pig, in which one or more lobules were said to be mucous and the rest to be albuminous. But in the guinea-pig, and possibly in the other cases, the mucous lobule appears to be a separate gland (cf. above, p. 476). It would probably be more convenient to use the term "mixed," for a gland in which the mucous and albuminous cells are present in approximately equal proportions. And we might have the following scale, passing from entirely albuminous to entirely mucous:—albuminous glands,

[1] "Traité de physiol. comparée des animaux," 3rd edition, 1886, tome i.

muco-albuminous glands, mixed glands, albumino-mucous glands, demilune glands, mucous glands.

The demilune cells, there can be little doubt, are albuminous secretory cells.[1] If we compare a series of submaxillary glands, passing from albuminous to mucous, we find that the albuminous cells become more and more confined to the ends of the terminal tubes, and the fewer there are the more compressed they become by the mucous cells, a feature, however, which is more marked in hardened than in fresh glands. And when the gland secretes, the demilune cells show obvious signs of secretory activity. The small discrete granules in them diminish in number, and in some animals form an inner granular zone; in alcohol-hardened glands, the demilune cells stain more deeply with carmine, and the nuclei and nucleoli are more conspicuous. The cells diminish in size after prolonged activity of the gland. In the earlier stages of secretion they appear to be larger, but this is probably due to a diminution in the size of the mucous cells, and so of the whole tube, whereby the demilune cells are less flattened.

A comparison of the large salivary glands in different mammals shows that the parotid has least variation in structure, and the submaxillary gland the most.

The parotid gland, as a rule, contains albuminous cells only; but in the dog there are commonly, if not always, a few mucous cells, or mucous alveoli present. And there may be in the dog a small mucous lobule, pouring its secretion into the duct a short distance from the main gland.

The submaxillary glands are entirely albuminous in rodents. In primates, the majority of the cells are albuminous, but some are mucous. In solipedes and ruminants, the glands are "mixed," but most of the cells are mucous. In carnivora the great majority of the alveolar cells are mucous, but some are albuminous, and there are fewer albuminous cells in the submaxillary gland of the dog than in that of the cat; thus in a microscopical preparation of the gland of a dog, the albuminous cells are almost entirely in the form of demilunes, whilst in a similar preparation of the gland of a cat, a considerable number of albuminous alveoli are seen. In the mole, large portions of the submaxillary gland do not even contain demilunes, and in these portions none but typical mucous cells occur.

The sublingual gland in all animals contains a greater or less proportion of mucous cells, and it is in consequence generally called a mucous gland. But as it always contains albuminous cells also it belongs properly to the class of mixed glands. The sublingual gland has certain characters which distinguish it from the submaxillary gland. It is more obviously tubular, the lumina are often large, the cells in a section of a hardened specimen are more columnar, and a considerable number of them consist, in their ordinary resting state, of proteid material in the outer third, half, or even two-thirds, and of mucous material in the remaining portion next the lumen.

The orbital gland[2] of the dog is mucous, the mucous cells are large and contain very little proteid substance, the demilunes are much flattened. The

[1] Langley, *Trans. Internat. Med. Cong.*, 1880; *Proc. Roy. Soc. London*, vol. xi. p. 364; and this view has been taken by most subsequent observers.

[2] Cf. Lavdowsky, *Arch. f. mikr. Anat.*, Bonn, 1877, Bd. xiii. p. 288.

admaxillary glands (Klein) have been found, so far as they have been investigated, to be mucous glands.

One or two points with regard to the structure of the alveolar cells, which bear upon questions we have to consider later, we may also mention.

In all salivary alveolar cells there are found, though with very different degrees of distinctness, more or less spherical granules, destined in an altered or unaltered condition to become part of the secretion. Whether the cell substance, in which the granules are embedded, has or has not a definite structure, we cannot decide with certainty; this cell substance may be what we speak of as granular structureless protoplasm; or it may consist of two parts,—a protoplasmic part forming externally a boundary layer, except perhaps towards the lumen, and internally a delicate network; and another part between the network and the granules, which in some cells may be of an albuminous and in others of a mucous nature.

Every gland has its own distinctive histological features, implying a distinctive chemical character in the substance it secretes. In addition, secreting cells of obviously different nature often occur in the same gland. Thus, in the submaxillary gland of the rat, there is an ordinary albuminous portion, and running through this are tubes, in bold curves, consisting of cells with large granules, which sometimes leave an outer clear zone. And in the submaxillary gland of the rabbit, the first cells of the alveoli, and the terminal ductules, have in the fresh state conspicuous granules, differing widely from the faint granules of the rest of the cells in the alveoli.

Origin and Course of the Nerves to the Salivary Glands.

All the salivary glands are supplied with nerve-fibres from two sources. They receive nerve-fibres, on the one hand, from the medulla oblongata, by way of some cranial nerve; and, on the other hand, from the spinal cord, by way of the cervical sympathetic.

The cranial nerve contains many, the sympathetic nerve comparatively few, secretory fibres. The cranial nerve contains vaso-dilator, and the sympathetic nerve contains vaso-constrictor fibres for the small arteries of the glands. There is, at present, no evidence worth considering that the cranial nerves have vaso-constrictor fibres for the glands, or that the sympathetic nerve has vaso-dilator fibres for them.

The chorda tympani and the nerve-cells with which it is connected.—The submaxillary, the sublingual glands, and the glands of the tongue, receive secretory and vaso-dilator fibres from the chorda tympani. The chorda tympani arises from the seventh nerve; it leaves this in the Fallopian canal, runs across the tympanum, and joins the lingual branch of the third division of the fifth nerve. The nerve thus formed may be called the *chordo-lingual*; it extends roughly up to the dorsal edge of the sublingual gland; here nearly all the fibres for the submaxillary gland, and about half of those for the sublingual gland, leave the lingual fibres, generally in four or five delicate strands, lying close together. These strands, with the tissue around them, are easily dissected out as a single bundle, and the bundle of nerve strands is called the chorda tympani, although it is a part only of the chorda tympani proper. The chorda tympani curves backwards towards the

gland ducts, and accompanies them into the glands. Other fine filaments, coming from the chorda tympani proper, are given off from both sides of the lingual, as it runs forward over the sublingual gland; most of these end in this gland, but a few fibres, varying in number in different animals, run back and supply the submaxillary gland. Finally, a few fibres, from the chorda tympani proper, continue their course in the lingual, and supply the glands and blood vessels in the area of distribution of the lingual nerve in the tongue.

On the course of the nerve filaments to the glands are a number of small and often microscopic ganglia. In the smaller filaments these begin a very short distance from the lingual nerve, and then occur at intervals as far as the terminations of the ducts. Fibres from the filaments and ganglia intermingle, and form a plexus; this plexus, at first, overlies the sublingual gland, but, further on, surrounds and accompanies the ducts, chiefly those of the sublingual gland. The larger part of the chorda tympani passes by this plexus, and runs direct to a ganglion in the hilus of the submaxillary gland; where the duct begins to divide, this ganglion gives off strands, which form another plexus, surrounding and accompanying the divisions of Wharton's duct.

Some of the ganglia in the plexus over the sublingual gland are relatively large; thus in the dog there is, as a rule, a ganglion, which may be seen with the eye, in the angle between the lingual and the chorda tympani. This was called, by Bernard, the submaxillary ganglion; as we shall see presently, it is more appropriate to call it the sublingual ganglion. Another, and a larger ganglion, is that spoken of above, as present in the submaxillary gland. As this belongs chiefly, if not entirely, to the submaxillary gland, we may call it the submaxillary ganglion. But it must be remembered that the nerve-cells which occur on the course of the chorda tympani fibres, either to the sublingual or to the submaxillary gland, are not collected together in a single ganglion, but occur scattered at intervals on the nerve-plexus into which the fibres run.

The nerve-cells are on the course, both of the secretory and of the vaso-dilator fibres of the chorda tympani. This may be shown by stimulating the chorda tympani, centrally of the nerve-cells, and peripherally of them, before and after injecting nicotine into a vein.[1] The experiment is best made in a cat. Normally, stimulation of the chorda tympani, in any part of its course, causes a flow of saliva, and an increased blood flow from the gland vein. After injecting a small dose of nicotine into a vein (cf. p. 515), stimulation of the chorda tympani in the tympanic cavity, or of the chordo-lingual nerve, has no effect. But a rapid secretion, and a greatly increased blood flow from the gland vein —in fact, the usual effects of stimulating the chorda tympani—are readily obtained by stimulating the nerve plexus in the hilus of the gland. Since no nerve except the chorda tympani is able to produce these effects, we may safely conclude that, in stimulating the nerve-plexus in the hilus of the gland, it is the peripheral chorda tympani fibres which cause the secretion and increased blood flow. The amount of nicotine given does not prevent—nor, so far as we know, affect—the passage of a nervous impulse along a nerve-fibre, and, in consequence, we conclude that the nerve-cells are on the course of the chorda

[1] Langley, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 123.

tympani fibres, and that nicotine either acts on the connection of the nerve-fibres with the nerve-cells, so that a nervous impulse cannot pass from one to the other, or acts on and paralyses the cells. The former is the more likely, and, in accordance with what is known generally regarding the relations of different nerve units, we may suppose that the nerve-fibres divide into fibrils, which terminate on the nerve-cells, and that these terminations are paralysed by nicotine.

I have only spoken, so far, of the effect, after nicotine injection, of stimulating the chordo-lingual and the plexus in the hilus of the gland. If the stimulus be applied between these two places, the effect varies in different cases, and varies also in different animals. Broadly speaking, as the electrodes are passed along the chorda tympani and nerve-plexus, towards the hilus, a point will be found where the stimulus causes a slight secretion; as the electrodes are passed more peripherally, the secretion increases, but it is rarely considerable, until the hilus of the gland is reached. This means that a few of the nerve-cells, outside the submaxillary gland, send their axis-cylinder processes to the gland. It may be mentioned that the relative number of these is greater in the rabbit than in the cat, and greater in the cat than in the dog.

Similar conclusions as to the relation of the fibres of the chorda tympani to the peripheral nerve-cells may be deduced from the experiments in which the chorda tympani has been cut, and time allowed for its fibres to degenerate. I shall deal later with these experiments (p. 519); and it will be sufficient to give here the chief result which bears on the question before us. When the chorda tympani, or the chordo-lingual nerve, is cut in a dog or cat, and the peripheral cut end, after about four days, is stimulated, no effect is produced; but if a little pilocarpine be injected, a fairly copious secretion is obtained, and the blood flow through the gland is increased. Although it is not agreed on all sides that pilocarpine produces a secretion by stimulating the nerve-endings in the glands, this is probably the case, and if it be so, the experiment shows that the chorda tympani fibres have degenerated up to the peripheral nerve-cells, whilst the fibres given off by the nerve-cells are still intact.

The position of the nerve-cells on the course of the chorda tympani fibres to the sublingual gland can similarly be determined. If a sufficient dose of nicotine be given to a dog, stimulation of the chordo-lingual nerve has no effect; stimulation of the ganglionic nerve-plexus, lying in the angle between the chorda tympani and the lingual nerve, causes constantly some secretion from the sublingual gland, but none, as a rule, from the submaxillary gland. The ganglion, called by Bernard the "submaxillary" ganglion, is the chief ganglion of this plexus; it follows, from what has just been said, that, at least in the dog, this ganglion sends fibres to the sublingual gland, but commonly sends no fibres to the submaxillary gland. It is, then, more accurate to speak of it as the sublingual ganglion.

It is well known that there are small groups of nerve-cells in the tongue itself; these, for the most part, are probably on the course of the chorda tympani fibres to the glands, and to the small arteries of the tongue, but there is no experimental evidence on the point.

The fibres of the chorda tympani pass through the geniculate

ganglion, but it is probable on general grounds that they are not connected with the nerve-cells of this ganglion.

The nerve-strands which leave the chordo-lingual and the lingual nerve to run to the sublingual and submaxillary plexuses consist in very large part of small fibres, about 2 μ to 3·5 μ in diameter,[1] but a few larger up to 8 or 10 μ are also present. In the plexuses the number of medullated fibres decreases, and the number of non-medullated fibres increases in passing towards the periphery. The axis-cylinder processes, then, of most, if not of all, the peripheral nerve-cells are non-medullated fibres.

The large nerve-fibres may occasionally be seen to divide. They are probably sensory fibres for the gland arising from the fifth nerve. Some of the small fibres may also be sensory.

Cranial nerve-fibres to the parotid and orbital glands.—The course of the secretory and vaso-dilator fibres to the parotid gland varies in different animals.

In the dog they arise from the ninth nerve ; they run—as Jacobson's nerve—across the tympanic cavity over the promontorium forming part of the tympanic plexus. From the tympanic cavity they proceed to the small superficial petrosal and otic ganglion, and thence to the auriculo-temporal branch of the fifth nerve, and so to the parotid gland.

In the sheep and ox the origin of the secretory fibres from the medulla is not known. They run in the buccal branch of the fifth nerve, instead of in the auriculo-temporal, leave this at the anterior end of the masseter muscle, and run backwards to the parotid gland along the duct.[2]

There are no experimental investigations on the place of connection with nerve-cells of the cranial fibres to the parotid gland, but it has been supposed that this connection occurs in the otic ganglion. No ganglion cells have been described in the parotid gland itself.

The secretory fibres for the orbital gland of the dog run in the buccinator branch [3] of the fifth nerve, and this is all that is known of their course.

Historical.—The history of the discovery of the course taken by the cranial secretory fibres [4] may be briefly summarised as follows :—

In 1851, Ludwig discovered in the dog secretory fibres for the submaxillary gland in the lingual branch of the fifth nerve. Rahn (and Ludwig) obtained in the rabbit secretion from the parotid, and sometimes from the submaxillary gland, on stimulating certain cranial nerve roots, after removing the brain. They found the effective nerve-roots to be those of the seventh and of the fifth, but their experiments do not show satisfactorily that the secretory fibres leave the medulla by way of these nerve roots.

Bernard showed that the secretory fibres of the submaxillary glands came from the chorda tympani and so from the facial nerve. That the chorda tympani had some connection with the flow of saliva from the submaxillary

[1] Cf. Heidenhain, *Arch. f. Anat. u. Physiol.*, Leipzig, 1883, Supp. Bd., S. 158 ; Gaskell, *Journ. Physiol.*, Cambridge and London, 1886, p. 29.

[2] Moussu, *Arch. de physiol. norm. et path.*, Paris, 1880, p. 68. (Cf. this paper also for secretory nerves of horse and pig.) Eckhard, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893.

[3] For the method of dissection for experimental purposes, see Heidenhain, Hermann's "Handbuch der Physiol.," Bd. v. Th. 1, S. 38.

[4] Ludwig, *Ztschr. f. rat. Med.*, 1851, N. F., Bd. i. S. 255 ; Rahn, *ibid.*, S. 285 ; Schiff, *Arch. f. physiol. Heilk.*, Stuttgart, 1851, Bd. x. S. 581 ; Bernard, "Leçons sur la physiol. et la

gland was suggested before Ludwig's discovery of secretory nerves, and was definitely stated by Schiff in 1851.

The course of the nerve-fibres to the parotid gland was also investigated by Bernard. He obtained secretion in the dog by stimulating the auriculo-temporal branch of the fifth nerve, and a cessation of reflex secretion by extirpation of the otic ganglion. He considered that the secretory fibres came from the small superficial petrosal nerve, and that the superficial petrosals and the chorda tympani arose from the nervus intermedius of Wrisberg. Abolition of the reflex secretion in the rabbit was observed by Schiff on simple section of the small superficial petrosal. Loeb found that section of the tympanic branch of the glosso-pharyngeal nerve (*i.e.* of Jacobson's nerve), or of the roots of this nerve in the skull, also abolished the reflex secretion, so that the secretory fibres of the small superficial petrosal come from the ninth and not from the facial. And Heidenhain obtained copious secretion on stimulating Jacobson's nerve.

If the secretory fibres of the parotid really arise from the ninth nerve, the majority of the early observations form a singular record of inadequate experiments and hasty deductions.

The sympathetic nerve-fibres and the nerve-cells with which they are connected.—All the salivary glands receive nerve-fibres from the cervical sympathetic. The fibres run from the middle or from the lower part of the superior cervical ganglion to the external carotid artery, and accompany its branches. On the arteries they form a plexus having two main longitudinal strands. The nerve-plexus, though chiefly of non-medullated fibres, contains some medullated fibres. In the artery to the submaxillary gland of the dog, there are twenty to thirty medullated fibres, a few of these being 5 μ to 7 μ in diameter, the rest 2 μ to 3·5 μ; the fibres run past the submaxillary ganglion in the hilus, without being, so far as can be seen, connected with it.

The sympathetic fibres both secretory and vasomotor, for the submaxillary gland of the dog and cat, arise chiefly from the second thoracic nerve, to a less extent from the third, fourth, and to a slight and varying extent from the first and fifth thoracic nerves.[1]

Langendorff[2] found that four months after hemisection of the spinal cord in the upper cervical region, the cervical sympathetic presented its normal appearance. We may conclude, then, that the glandular nerve-fibres do not descend from a secretory centre in the medulla, and simply make their exit by the upper thoracic nerve roots.

And there are several grounds for believing that the efferent sympathetic nerve-fibres issuing from a particular nerve root are the axis-cylinder processes of nerve-cells situated in the corresponding segment of the spinal cord.

pathol. du système nerveux," 1858, tome ii.; Schiff, "Lehrbuch. d. Muskel. u. Nervenphysiologie," 1858–1859, S. 393; Czermak, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1860, Bd. xxxix. S. 526; *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1860, Bd. ii. S. 214; 1863, Bd. iii. S. 49; Navrocki, *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1865, Heft 4, S. 123; Loeb, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1869, Bd. v. S. 1; Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 15; Bernard, "Leçons de physiol. opératoire," 1879.

[1] Langley, *Phil. Trans.*, London, 1892, vol. clxxxiii. p. 104.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 165. Strictly speaking, the experiment only shows that the great majority of the nerve fibres of the cervical sympathetic have their trophic centre in the spinal cord below the hemisection. If even a considerable number of fibres had degenerated, they would have been absorbed in the time allowed, and would have left no recognisable trace.

The sympathetic nerve-fibres are connected with nerve-cells in the superior cervical ganglion. If the cervical sympathetic be cut, the end towards the ganglion gives, in about four days, no effect on stimulation, but stimulation of the ganglion itself or of the fibres beyond it causes secretion and pallor of the gland (p. 522). On microscopical examination, the nerve-fibres are found to be degenerated, as far as the ganglion but not beyond it. Injection of nicotine causes for a time, varying with the dose, effects like those caused by degeneration of the nerve.[1] In the cat even 5 mgrms. of nicotine may be sufficient to paralyse the cervical sympathetic for a time, but very large amounts, *e.g.* 500 mgrms., do not paralyse the nerves beyond the ganglion. From this and from other facts we deduce that the sympathetic fibres are not connected with any sympathetic nerve-cells peripherally of the superior cervical ganglion; and there are reasons for believing that they are not connected with any nerve-cells between the ganglion and the spinal cord. In the dog the cervical sympathetic is much less readily paralysed by nicotine.

Secretion of saliva produced by stimulation of the medulla oblongata.—Bernard[2] found that puncture of the fourth ventricle in the dog causes secretion from all the salivary glands, and if the puncture be a little above the spot, injury of which produces diabetes, the secretion may be confined to the submaxillary gland, and from this gland may be abundant. Loeb[3] showed that puncture of the medulla caused a greater secretion from the submaxillary or the parotid gland, according as the puncture was in the region of the nucleus of the ninth or of the seventh nerve respectively. With puncture on one side, the effect on the submaxillary gland of the opposite side was much greater than on the parotid of the opposite side. Grützner and Chtapowski[4] observed that stimulation of the medulla oblongata caused abundant secretion if the chorda tympani was intact, a slight secretion if it was cut, but none after section of both the chorda and the sympathetic.

Secretion of saliva produced by stimulation of the cerebral cortex.—It is not clear that the cortex of the cerebral hemispheres is connected with secretion—or indeed with any visceral phenomenon—in the way in which it is connected with the various body movements.

Stimulation of the motor area, taking the matter broadly, causes secretion from the salivary glands, more readily than does stimulation of any other part of the cortex. So far as the experiments go, the region which causes maximum secretion from the submaxillary gland causes also maximum secretion from the parotid. Apparently the secretion ceases on cutting the cranial secretory nerve.

The experiments in which the portions of the cortex which cause secretion have been mapped out were made on dogs under curari. Those who have experimented on undrugged animals find that stimulation of the facial area causes no secretion so long as the resulting movement is confined to the facial muscles, and Eckhard[5] states that the secretion of saliva from the submaxillary glands only begins when the stimulus is continued long enough, or is made strong enough, to induce

[1] Langley and Dickinson, *Proc. Roy. Soc. London*, 1889, vol. xlvi. p. 425; Langley, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 131.

[2] "Leçons de physiol. expérimentale," 1856, Bd. ii.

[3] *Op. cit., supra.*

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii. S. 522.

[5] *Neurol. Centralbl.*, Leipzig, 1889, p. 65. Cf. also *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1876, Bd. vii S. 199.

general convulsions; he considers that the saliva obtained in the curarised animal is due to an irradiation of nervous impulses, and not to a localised cortical stimulation.

Külz[1] obtained no secretion from the submaxillary gland in unanæsthetised dogs on stimulating the facial area, unless there was general tetanus, a condition in which Braun[2] had already observed a flow of saliva from the mouth. Lépine and Bochefontaine[3] obtained secretion in curarised dogs by stimulating the anterior portion of the cortex, including the facial area. The secretion was more abundant on the side stimulated. Bochefontaine,[4] shortly after, gave a more detailed account of the parts of the cortex from which secretion could be induced; secretion was obtained by stimulating spots on the posterior part of the brain, and also by stimulating parts of the dura mater. The experiments show little or nothing as regards the question whether there are special areas in the cortex connected with the secretion.

Bechterew and Mislawsky[5] found, also on curarised dogs, that the region which caused secretion when stimulated with weak currents was more limited than that described by Bochefontaine. Stimulation of the anterior Sylvian and anterior composite convolutions caused secretion from both the submaxillary and parotid glands. Stimulation of the anterior limb of the sigmoid gyrus, and of the anterior extremities of the coronal and anterior ecto-Sylvian convolutions, caused secretion from the submaxillary gland only. With stronger currents, secretion was sometimes obtained from the more posterior portions of the cortex. They found, unlike Lépine and Bochefontaine, no effect on stimulating the orbital convolution.

Changes in Salivary Glands during Secretion.

The changes which occur in salivary glands during secretion are progressive, and there is no sufficient reason for believing that the changes which occur in the cells at the end of a day's active secretion differ in kind from those which occur in the first ten minutes.

The evidence is, it seems to me, decisively against the view that during salivary secretion there is a breaking down of the mucous or of other gland cells.[6] If saliva at any stage of secretion is allowed to run into alcohol, mercuric chloride, or other hardening reagent, disintegrating cells are not seen in the sediment as it forms, nor nuclei beyond those which arise from the separated cells of Wharton's duct and from leucocytes. And in the gland itself there is at no stage any sign of active cell division; the nuclei undergoing mitotic division are as rare as they are in the resting gland.[7]

Two fundamental changes undoubtedly take place in the gland cells during secretion.

There is, first, an excretion of a greater or less amount of the substance which has been previously formed in the cells;[8] this substance,

[1] *Centralbl. f. d. med. Wissensch.*, Berlin, 1875, S. 419.
[2] *Beitr. z. Anat. u. Physiol. (Eckhard)*, Giessen, 1876, Bd. vii. S. 136.
[3] *Gaz. méd. de Paris*, 1875, p. 332.
[4] *Arch. de physiol. norm. et path.*, Paris, 1876, p. 161.
[5] *Neurol. Centralbl.*, Leipzig, 1888, p. 553.
[6] It must be mentioned, however, that Heidenhain in his treatise (Hermann's "Handbuch," 1890, Bd. v.) maintains the view originally advanced by him, that mucous cells disintegrate to form part of the secretion.
[7] Langley, *Proc. Roy. Soc. London*, 1886, vol. xli. p. 362; Bizzozero, *Virchow's Archiv*, 1887, Bd. cx. S. 181.
[8] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 43; Hermann's "Handbuch," 1883; Lavdowsky, *Arch. f. mikr. Anat.*, Bonn, 1877, Bd. xiii. S. 335.

as it is formed, is for the most part, at any rate, stored up in the cells in the form of granules.[1]

Secondly, there is a taking up of proteid material by the cells.[2] This occurs more or less exclusively in the outer part of the cells, and is the chief cause of the formation of an outer non-granular zone.[1] The taking up of fresh proteid substance is usually spoken of as a growth of protoplasm; we may use the expression as a matter of convenience, bearing in mind that a large portion of the fresh proteid substance may be simply deposited in interstices or larger spaces of the protoplasm.

It is probable also that, during the whole period of secretion, there is a conversion of the newly taken up proteid into the material for secretion, or, in other words, the protoplasm is continuously disappearing and giving rise to granules.

The loss of granules, together with the growth of protoplasm, causes the gland to become less white and less opaque to the eye.

The nucleus, as was first shown by Heidenhain, is more obvious, and the nucleolus more conspicuous, in sections of the active gland than in those of the resting gland. The shrunken state of the nucleus in the resting gland appears to be due to the action of the hardening agent, for in teased glands, when the nucleus is visible, without serious alteration in the normal form of the cell, it is seen to be spherical. Nevertheless it is probable that there is some increase in the organic substance of the nucleus during prolonged secretion.

During rest [3] the protoplasm decreases and the granules increase, and it would not be unnatural to suppose that no other changes take place in the cells but those associated with the conversion of protoplasm into a substance ripe for excretion. The point is one of great importance for the proper understanding of the secretory processes. Is there or is there not during rest any interchange between the cells and the lymph, beyond that of the taking up of oxygen and the giving off of carbonic acid? There is no experimental evidence to show whether the amount of oxygen taken up by the gland cells is so much in excess of the carbonic acid given off, that an increase in the weight of the gland takes place; but this is on general grounds probable enough, to prevent us from attributing offhand any increase in weight which may occur in a gland during rest to its cells having taken from the lymph proteid or substance other than oxygen.

We may take first the evidence that glands increase in weight during rest. Obviously this is proved, if it can be proved that there is a decrease in weight during secretion.

Heidenhain stimulated the chorda tympani on one side in a dog, and, after obtaining a considerable amount of saliva, killed the animal by bleeding it, separated the glands on the two sides from their capsules, and as far as possible from connective tissue, and then weighed them. He found that the active gland weighed less than the resting gland.

[1] Langley, *Journ. Physiol.*, Cambridge and London, vol. ii. p. 261; *Internat. Monatschr. f. Anat. u. Histol.*, 1884, vol. i. p. 69; *Proc. Roy. Soc. London*, 1886, vol. xi. p. 362.

[2] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 43; Hermann's "Handbuch," 1883; Lavdowsky, *Arch. f. mikr. Anat.*, Bonn, 1877, Bd. xiii. S. 335.

[3] It is perhaps hardly worth while to defend the use of the word "resting" for a gland which for some time has secreted but little, and the use of the word "active" for a gland which for some time has been secreting more or less copiously. The words lead to no ambiguity, and the objections to them appear to me purely pedantic. "Active" and "resting," applied to any living tissue, are essentially relative terms; it can hardly be doubted that there is greater chemical change when secretion is going on than when it is not.

Three experiments were made; the chorda tympani was stimulated on the left side.

	Amount of Saliva obtained.	Weight of Active Gland.	Weight of Resting Gland.
Experiment 1 . .	55 c.c.	5·04 grms.	5·06 grms.
,, 2 . .	75 c.c.	5·42 ,,	6·86 ,,
,, 3 . .	220 c.c.	5·91 ,,	6·36 ,,

In these experiments the left gland was the one that was caused to secrete, and there is some reason to think that the left gland is normally heavier than the right, for Bidder found this to be the case in eleven cases, and Heidenhain in two.[1] Pawlow,[2] however, noticed no appreciable difference in the amount of nitrogen contained by ten right and by ten left submaxillary glands of the dog. But so long as it is not shown that the right gland may be normally heavier than the left, we may fairly conclude that there is a loss of weight by the gland during secretion and a gain of weight during rest.

The question may be approached from another side. Microscopical examination shows decisively that during secretion the gland cells become smaller; they must then, taken together, decrease in weight unless the percentage of solids in them increases. But, according to Heidenhain, the percentage does not increase during secretion; on the contrary, it decreases. Thus, in one experiment upon a dog, in which about 220 c.c. of saliva were obtained by stimulating the chorda tympani, the percentage of solids in the resting gland was 28·3 per cent., and in the stimulated gland it was only 21·3 per cent., so that there were 7 per cent. less solids on the stimulated side.

This, it must be remembered, applies to the gland as a whole. In concluding that there is a decrease in the percentage of solids in the actual gland cells, we assume that the percentage composition of the glands on the two sides is approximately the same, that there is no appreciable difference in the amount of blood and lymph upon the two sides, and that the connective tissues in the gland are too small in amount or too constant in composition to affect the result; these assumptions, however, appear to be justifiable.

The other experiments made by Heidenhain [3] were as follows:—

1. The left chorda tympani was stimulated and 75 c.c. of saliva obtained. The right submaxillary gland contained 23 per cent. of solids, the left gland 18·6 per cent.—a decrease of 4·4 per cent.

2. The left chorda tympani was stimulated and 55 c.c. of saliva obtained. The right submaxillary gland contained 24 per cent. of solids, the left gland 21·5 per cent.—a decrease of 3·5 per cent.

Heidenhain found a slight decrease also in the percentage of solids on stimulating the cervical sympathetic.[4] The time of stimulation is given, but not the amount of saliva obtained.

[1] *Op. cit.*, p. 57.
[2] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1888, S. 137.
[3] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, Heft 4, S. 55.
[4] *Op. cit.*, p. 66.

1. Sympathetic stimulated for two and a half hours. The resting gland had 25 per cent. of solids, the stimulated gland 23·6 per cent.

2. Sympathetic stimulated for five and a half hours. The resting gland had 25 per cent. of solids, the stimulated gland 24·4 per cent.

We may conclude, then, that during secretion the gland cells decrease in weight, and therefore that they increase in weight during rest. The increase in rest might be due, as we have said, to a taking up of oxygen. But the observations of Pawlow,[1] if they are well founded, show that, whether an increase in weight due to oxygen combinations occurs or not, there is during rest a not inconsiderable increase in the nitrogen of the glands, and this can hardly be due to anything else than an absorption of proteids. Pawlow estimated by Kjeldahl's method the amount of nitrogen in the resting submaxillary gland of the dog on the one hand, and in the stimulated gland and in the saliva secreted by it on the other hand. He obtained saliva by stimulating the central end of the sciatic for one and a half to five hours. In ten (right) stimulated glands he found 1·872 grms. nitrogen. In the ten (left) non-stimulated glands he found 2·18 grms. of nitrogen. Assuming, then, that the glands had the same amount of nitrogen to start with, the stimulated glands had lost during secretion about $\frac{1}{7}$ of their nitrogen-holding substance. In the saliva secreted he found 0·416 grms. of nitrogen, so that presumably the glands had taken up during secretion about 0·1 grm. of nitrogen; this is about $\frac{1}{21}$ of the total amount. The numerical results are not such as we should expect from the microscopical appearances of the gland cells, and it is desirable that the experiments should be repeated.

The general characters of the cells of the lobular ducts suggest that they are not simply the lining cells of a conducting tube, but are rather active constituents of the gland, concerned either with adding to the saliva as it passes by them, or with subtracting from it. There is not, however, any clear evidence on the matter. It is true that when a considerable amount of methylene-blue is injected into the blood, and the glands are excited to secrete, small deep blue particles may be found in the duct cells as in the alveolar cells, but methylene-blue is so readily taken up by many tissues that little trust can be placed on this as showing a secretory function. The cells of the lobular ducts contain small granules in their outer portion,[2] and, according to Mislawsky and Smirnow,[3] these granules decrease during secretion, but it does not appear to me certain that the changes described by these authors are not due to conditions other than secretory activity. Merkel[4] observed that the cells with striated epithelium, *i.e.* most of the lobular duct cells of the submaxillary and parotid glands, stained a deep brown when treated with pyrogallic acid in the presence of oxygen. He considered that the stain was due to the presence of calcium salts in the cells. This naturally suggested that the lobular ducts with striated epithelium might secrete calcium salts. But Werther[5] has shown that the percentage of calcium salts in the sublingual saliva of the dog is rather greater than in

[1] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1888, S. 137.

[2] Langley, *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 433. When the granules of the duct cells swell up and become indistinct, the substance between them takes on the characteristic striated appearance seen in hardened specimens.

[3] *Arch. f. Anat. u. Physiol.*, Leipzig, 1896, Physiol. Abth., S. 93.

[4] "Die Speichelröhren," Leipzig, 1883.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 293.

submaxillary saliva. And it happens that the ducts with striated epithelium are very scanty in the sublingual gland, whilst they are numerous in the submaxillary gland.

Reflex Secretion of Saliva in Normal and in other Conditions.

In man, more complete observations have been made on the flow of saliva from the parotid than on that from the submaxillary gland, since the duct of the parotid is sometimes accidentally injured, so that the establishment of a parotid fistula becomes necessary. But some of the conditions of flow from either gland may be readily observed, when a cannula is simply placed in the opening of the duct of the gland into the mouth.

In the dog, sheep, horse, and other animals, sometimes a permanent fistula, and sometimes a temporary fistula, of one or more of the glands is established. The observations have been made with and without the administration of anæsthetics.

Ordinarily, between meals, the large salivary glands—except the parotid glands of ruminants—do not secrete. But as the mucous membrane of the mouth is constantly kept moist, saliva must constantly be formed by the smaller glands of the mucous membrane. In some animals the amount of this secretion is very considerable; thus in the horse, during abstinence, 100 to 150 c.c. of saliva are, according to Colin,[1] formed in an hour. Probably during sleep the amount diminishes. There is little doubt that this secretion is produced reflexly by conditions affecting the mucous membrane of the mouth, and a slight increase in the strength of the stimuli probably sets in action the larger glands also.

In ruminants there are some peculiarities. The parotid gland secretes continuously (Colin,[1] Eckhard [2]). The secretion is most abundant during feeding, rather less during rumination, and one-eighth to one-fourth the rumination rate during rest.[1] During rest, the submaxillary glands secrete little or not at all, and it is a remarkable fact that rumination does not, as a rule, cause any secretion from these glands, although it increases the secretion from the parotid gland, and although feeding causes a secretion from all the glands.

Colin found in ruminants a slight continuous secretion from the submaxillary and sublingual glands during rest. Ellenberger and Hofmeister[3] found none, but they noticed that there was occasionally a slight secretion from the submaxillary gland during rumination, and a more copious secretion during the act of drinking. According to these observers, there are occasional short pauses in the parotid secretion during rest.

In ruminants, further, it has been said [4] that the secretion from the parotid gland continues after section of all the nerves running to it. In the ox, Moussu (1890) found that section of the buccal nerves diminished greatly, but did not quite stop, the parotid secretion. Eckhard (1893) states that section of these nerves does not affect the parotid secretion in the sheep; he found about $1\frac{1}{2}$ c.c. to be secreted in ten minutes, whether the nerves were cut or no. The matter requires further investigation.

[1] *Op. cit.* [2] *Ztschr. f. rat. Med.*, 1867, Bd. xxix. S. 74.
[3] *Arch. f. Anat. u. Physiol.*, Leipzig, 1887, Physiol. Abth., Supp. Bd., S. 138.
[4] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, S. 365. Cf. Schwann, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, Bd. vii. S. 170.

In man during hunger, the sight, smell, or idea of food is sufficient to cause a secretion of saliva from all the salivary glands; and chewing insoluble substances has a similar, though apparently a less effect.[1]

Secretion in this way is said not to occur in lower animals. Thus Schiff[2] found in a dog with a parotid fistula, that no flow of parotid saliva was caused by the sight or smell of the meat the animal was endeavouring to obtain; when it was induced to bite a piece of wood, the meat still being in sight, there was slight secretion from the submaxillary gland but none from the parotid, but on placing sapid substances in the mouth there was at once a rapid secretion. And Colin states that, after a parotid fistula has been established in a horse, and when the animal is in a state of hunger, there is no secretion from the parotid when the animal is offered, but not allowed to take, corn, nor when it masticates oakum, although mastication of corn readily causes a secretion.

Sapid substances taken into the mouth cause more or less secretion from all the salivary glands. In man all substances are effective, and drinking, wine for example, is sufficient.[3] Acid placed on the tongue is apparently the most effective stimulant among the sapid substances, but there are not sufficient observations in man as to the amount of saliva produced by other substances, to allow a satisfactory opinion to be formed as to the relative effectiveness of salt, sweet, and bitter bodies. Mastication considerably increases the flow of saliva, probably by bringing the particles into better and more frequent contact with the mucous membrane.

Chloroform and ether when inhaled cause secretion, by stimulating the gustatory nerve endings, and possibly also the other nerve-endings in the mucous membranes; if given by the trachea, they do not cause secretion. Alcohol, ether, or chloroform, when mixed with water and held in the mouth, cause a fairly free secretion of saliva.

In carnivora, so far as the experiments go, acids (vinegar, tartaric acid) cause the most abundant secretion; salts, either neutral or alkaline, a less secretion, but still a fairly copious one; bitter substances a much less secretion, and sugar little or even none. With sapid substances in the mouth the secretion is increased by mastication.

Thus Bernard,[4] in one experiment on a dog, in which cannulæ were placed in the ducts of all three glands, obtained a copious secretion from vinegar, less from sodium carbonate, still less from colocynth, and none from sugar or from water. The relative effect on the several glands was practically the same with all the substances.

Schiff[5] obtained some secretion from the parotid fistula of a dog by placing sugar on the base of the tongue, but none by placing it on the tip.

According to Colin,[6] weak acids, salts, or aromatic substances placed on the buccal mucous membrane give rise to no appreciable secretion from the parotid of the horse during abstinence, and do not sensibly increase the con-

[1] Colin and Prompt, 1874 (see Colin, "Traite de physiol. comparée," etc., 3rd edition, p. 1), in the case of a girl with a parotid fistula, noticed that chewing a piece of ribbon caused a secretion of only one drop of saliva in two minutes.

[2] "Leçons sur la physiologie de la digestion," 1867.

[3] Colin and Prompt (1874), case of parotid fistula (cf. *supra*).

[4] "Leçons de physiol. expér.," 1856, tome ii. p. 82.

[5] "Leçons sur la physiol. de la digestion," 1867, tome i. p. 186.

[6] *Op. cit.*, p. 653.

tinuous parotid secretion of ruminants; but do nevertheless cause a secretion from the submaxillary and sublingual glands. His statements, however, are not quite consistent, and we may suppose that the difference is only one of degree.

In herbivora, mastication is performed alternately on the two sides, the periods being usually one quarter to half an hour. In the horse, and probably in other herbivorous animals, the secretion from the parotid is much greater on the masticating than on the non-masticating side. In the horse two to three times as much saliva is usually secreted on the masticating as on the opposite side, but the ratio may be either greater or less than this (Bernard, Colin). It seems reasonable to suppose that this is due to the better contact of food with the mucous membrane of that side of the mouth. According to Colin, however, there is no such difference in the secretion of the submaxillary and sublingual glands.

The amount of saliva secreted varies with the nature of the food. In man the data are not sufficient to form an estimate of any value, either of the relative amount of saliva secreted from the several glands, or of the total amount secreted in twenty-four hours. It is generally supposed that the total amount exceeds a litre a day.[1]

Bernard[2] found that in the dog, when saliva was obtained reflexly, the submaxillary gland secreted about twice as much as the parotid, and about ten times as much as the sublingual. And these are approximately the relative amounts obtained by injecting pilocarpine. The amounts secreted are roughly in proportion to the respective weights of the glands.

In herbivora the volume of saliva secreted by the submaxillary and sublingual glands does not correspond with their respective weights. According to Colin, the parotid gland of the horse secretes fifteen to twenty times the volume of saliva secreted by the submaxillary gland, but is only about four times its weight. And in the ox the parotid secretes four to five times as much saliva as the submaxillary gland, though it is slightly less in weight (cf. Table, p. 477).

Colin estimates that in the horse the total quantity of saliva secreted in a day is about 40 litres.

The total quantity of saliva may be estimated in two ways—(1) By comparing the weights of a certain amount of food before and after mastication and swallowing, the food after mastication being collected from an œsophageal fistula; and (2) by noting in different experiments the quantity secreted by each gland during a given period of feeding. Colin found, by the first method, that a horse secreted 5000 to 6000 grms. of saliva in an hour, when fed with hay, one-half of this when fed with grass, and one-third more than this when fed with oats.

During digestion, according to Colin, the submaxillary gland of one side secretes 25 to 30 c.c. of saliva in fifteen minutes; the parotid secretes 500 to 1000 (about) in fifteen minutes, if mastication takes place on this side.[3]

In the ox he estimates that during three hours' mastication, and five hours' rumination, about 40 litres of saliva are secreted, and that 16 litres are secreted during the sixteen hours of rest.

Electrical excitation of the central end of the lingual or of the glosso-pharyngeal causes secretion from all the salivary glands. The secretion

[1] Some data are given by Tuczek, *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 534.
[2] "Leçons de physiol. expér.," 1856, tome ii. p. 82.
[3] For other observations on this point, cf. Ellenberger and Hofmeister, *op. cit., supra.*

is less copious than that obtained by placing acids in the mouth, and it is more copious on the side stimulated than on the opposite side.

A special relation has also been said to exist between the state of the mucous membrane of the stomach and the secretion of saliva. Thus it has been said that a secretion of saliva is induced by the contact of various substances with the gastric mucous membrane.[1] This, however, is not satisfactorily proved. Braun[2] observed a dog, in which a gastric fistula had been established, and a cannula placed in Wharton's duct. No secretion of saliva was caused by introducing into the stomach, flesh, acetic acid, ether, nor by irritating the mucous membrane with a sponge.

Stimulation of the central end of the vagus has rather variable results on the submaxillary secretion of the dog. It usually causes secretion after a long latent period, and the secretion may continue for some time after the cessation of the stimulus. Oehl[3] obtained secretion although the stimulation caused no vomiting or arrest of respiration; the secretion occurred from both glands, but was greater on the side stimulated. Buff, as a rule, only obtained secretion when there was some body movement.

Bernard noticed that a flow of saliva may be obtained by stimulating the sciatic[4] and various other sensory nerves; it may, indeed, be obtained by stimulating any sensory nerve in the body. This reflex secretion is abolished by deep anæsthesia; whether it ceases coincidently with the production of anæsthesia is, however, uncertain. According to Buff,[5] the secretion does not occur in uncurarised animals, unless the stimulus produces also a reflex body movement.

The gustatory reflex secretion is caused wholly by impulses passing down the cranial secretory nerves. But a secretion may, in certain circumstances, be caused by impulses passing along the sympathetic nerve; for example, when the central end of a sensory nerve is stimulated, the secretion, so far as is known, is always accompanied by a constriction of the blood vessels of the gland.

In man, cases sometimes occur in which there is a permanent absence of secretion from the large salivary glands, and from the glands of the mucous membrane of the mouth. Such cases are rarer in men than in women. In women the loss of secretory power usually comes on after middle life, and may be the result of an emotional shock. For some time pilocarpine will still cause a secretion of saliva (Hadden), but eventually it causes none, though it still causes sweating.[6] The absence of secretion is no doubt due to a derangement of the reflex nervous mechanism, so that impulses passing up the afferent nerves no longer give rise to efferent impulses. The lack of normal functional activity probably causes a gradual atrophy of the glands, and a diminution of irritability of the nervous and glandular structures, so that eventually pilocarpine—or the amount of it which can be given safely—no longer produces a flow of saliva.

[1] For an account of papers on the reflex secretion of saliva, cf. Buff, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1888, Bd. xii. S. 3.

[2] *Ibid.*, 1876, Bd. vii. S. 44.

[3] *Compt. rend. Acad. d. sc.*, Paris, 1864, tome ix. p. 336. Secretion on stimulation of the central end of the vagus was first observed by Bernard, 1859.

[4] Cf. also Owsjannikow and Tschiriew, *Mélanges biol. Acad. imp. d. sc. de St.-Pétersbourg*, 1872, tome viii. p. 651.

[5] *Op. cit.* [6] Hutchinson, cf. Hadden, *Brain*, London, 1889, vol. xi. p. 484.

The Dyspnœic Secretion.

At a certain stage of dyspnœa the saliva flows with considerable rapidity from all the salivary glands. The time at which it begins and its amount are dependent upon the degree of anæsthesia. In anæsthesia, the secretion does not usually begin until the stage of expiratory convulsions. With a large excess of anæsthetics, the animal may be killed by asphyxia without any secretion occurring, or with only a trifling amount. When a copious secretion occurs it is due to impulses passing down the cerebral nerve fibres, but some secretion may be obtained after section of these nerve fibres. In such case there is also contraction of the glandular arteries. Whether dyspnœa is capable of producing a secretion after section of the cerebral nerve and excision of the superior cervical ganglion, has not been sufficiently investigated.

Stimulation of the Cranial Nerve supplying a Salivary Gland.

On some general features of the secretion.—A flow of saliva can be obtained from any of the salivary glands by electrical, mechanical, or chemical stimulation of the cranial secretory nerves. It need hardly be said that the interrupted current is the most effective form of stimulus. A very weak interrupted current, which cannot be felt on the tongue, is sufficient to cause a secretion. Within certain limits the rate of flow of the saliva increases with the strength of the stimuli, but strong currents rapidly injure the nerve at the point of stimulation. Even with moderate currents a very slight shifting of the electrodes on the nerve usually causes a marked increase in the rate of secretion, a fact which it is important to bear in mind in collecting for analysis different samples of saliva, secreted under different conditions.

The flow of saliva with a moderate strength of current is very rapid; thus the submaxillary gland in the dog may secrete in five minutes an amount of saliva weighing as much as the whole gland.

The nerve can be stimulated electrically for half an hour to an hour, and probably with proper precautions very much longer, without the flow of saliva ceasing. Pilocarpine in successive doses (cf. p. 513) will cause a secretion for, so far as we know, an indefinite time.

In protracted electrical stimulation the maximum amount of saliva is obtained by stimulating for short periods, with short intervals of rest; the stimulation being stopped each time as the secretion becomes slow. In this way in ten to twelve hours about 250 c.c. of saliva can be obtained from the submaxillary gland of the dog, and a half to two-thirds of this amount from the parotid. The rate of flow gradually diminishes during the progress of the experiment. With a given strength of current, the maximum rate of secretion is produced with a rate of interruption of about forty a second.[1]

According to Wedensky, rapid shocks, such as 100 to 250 a second, cause a change in the nerve-endings, so that they soon cease to transmit nervous impulses. His most striking experiment is the following:—Two pairs of electrodes are placed on the chorda tympani, shocks of moderate rate are passed through the lower, and of rapid rate through the upper; the secretion

[1] Wedensky, *Compt. rend. Acad. d. sc.*, Paris, 1892.

soon becomes slow or stops altogether, but, on cutting off the rapid shocks from the upper electrodes, the stimuli at the lower electrodes become again effective, and the secretion starts once more. The results are similar to those he obtains with motor nerves to skeletal muscle.

The real latent period of the gland cells cannot be accurately determined by any direct method, and in consequence it is customary to speak of the interval between the moment of stimulating the nerve, and the moment at which the movement of saliva occurs in the duct, as the latent period. When the cranial nerve is stimulated with a weak current, there is an obvious interval—usually two to four seconds—between the moment of application of the stimulus and the appearance of saliva in the cannula, and this is the case although the secretion when it occurs is not scanty. When stronger currents are used, and the secretion is copious, the latent period is much diminished. On the other hand, when the secretion is scanty, the latent period is very much prolonged, whatever the strength of current; thus, after a small dose of atropine, it may be half a minute or even more.

The percentage of organic substance in saliva obtained from different salivary glands varies considerably; in each, as we shall see, it varies in different circumstances, and in each it may be small (0·2 to 0·5 per cent.). But, other things being equal, the submaxillary saliva has usually a higher percentage of organic substance than either the sublingual or the parotid saliva.

There is a curious difference in the percentage of salts found in different salivas. In the dog the maximum percentage of salts in the parotid saliva is about 0·68, in that of the submaxillary gland about 0·77, and in that of the sublingual gland about 1·0.[1] In the rabbit the parotid saliva has a maximum percentage of about 0·85.[2]

After action of a strong stimulus.—Strong stimulation of the cranial nerve alters the gland it supplies in such a way, that the saliva secreted shortly afterwards has a higher percentage of solids than it otherwise would have had.[3] Thus, in the experiment quoted on p. 501, the first weak stimulation of the chorda tympani caused secretion of a saliva containing 0·52 per cent. of organic substance, whilst, after a strong stimulation, a second weak one caused a secretion having a percentage of 1·07 of organic substance.

This, however, only holds when successive small quantities of saliva are collected; with larger quantities, as 10 c.c. to 12 c.c., no such after action is observed (Werther).

Stimulation of the Sympathetic Nerve supplying a Salivary Gland.

Ludwig[4] (in 1856) discovered the secretory power of the sympathetic; he obtained a secretion from the submaxillary gland of the dog, by stimulating both the cervical sympathetic and the nerve filaments on the gland artery.

[1] Werther, *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 293; Langley and Fletcher, *Phil. Trans.*, London, 1889, vol. clxxx. p. 109.

[2] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 40.

[3] Heidenhain, *op. cit.*, 1878.

[4] Quoted by Czermak, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1857, Bd. xxv. S. 3; Czermak also obtained secretion on stimulating the cervical sympathetic.

Eckhard[1] noticed that the saliva secreted by the submaxillary gland, on stimulation of the sympathetic, was more viscid and contained a higher percentage of solids than that obtained by stimulating the chordo-lingual.

Neither from the submaxillary, the sublingual, nor the parotid gland of any animal does the sympathetic produce a secretion which approaches in amount that which is produced by the cranial nerve. Unless the gland has been secreting under the influence of the cranial nerve, before stimulation of the sympathetic (cf. p. 496), this stimulation causes secretion of a few drops only, and it may be much less. Thus, in the dog, stimulation of the sympathetic for a minute will ordinarily produce two or three drops from the submaxillary gland, and perhaps half a drop from the sublingual.

In most of the earlier experiments upon the parotid gland of the dog, either no secretion was obtained by repeated stimulation of the sympathetic, or a total amount not exceeding a few drops. This is, however, only a more marked instance of the slow secretion which the sympathetic, after the first few stimuli, causes in the submaxillary and sublingual glands of the same animal. If the parotid gland, after sympathetic stimulation, during which no secretion or a trace only has been obtained, be hardened, and sections be cut, the lumina, ductules, and duct will be found distended with secretion.

The maximum total amount of saliva is obtained by stimulating the sympathetic for short periods, with short intervals of rest. Stimulated in this way—say, during every other half-minute—the sympathetic will give from the submaxillary gland of the dog $\frac{1}{30}$th to $\frac{1}{60}$th of the quantity of saliva that would be obtained by similar stimulation of the chorda tympani.

With protracted stimulation the secretion may continue slowly for several minutes, but sooner or later it stops. Roughly speaking, and within rather narrow limits, the amount of saliva obtained is inversely proportional to the duration of the previous stimulus and directly proportional to the length of the preceding period of rest. After repeated stimulation of the sympathetic, there may be no visible secretion for half a minute to a minute after the beginning of the stimulation, and occasionally the slight secretion which occurs only begins after the stimulation has ceased.

Heidenhain, stimulating for a quarter of an hour during each half-hour, obtained a secretion from each stimulation for eleven successive hours, *i.e.* as long as the experiment lasted.

In different glands, and in the same gland in different animals, the freedom of secretion of sympathetic saliva compared with that produced by the cranial nerve, and the percentage of organic substance in the saliva, varies considerably. I have already mentioned that the sympathetic causes some secretion from the submaxillary gland, and often none from the parotid. Relatively, rather more sympathetic secretion is obtained from the glands of the cat and rabbit than from those of the dog. The sympathetic saliva from the submaxillary gland of the dog contains 1 to 3 per cent. of organic substance, that from the

[1] Adrian and Eckhard, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1860, Bd. ii. S. 83. Bernard, *Journ. de l'anat. et physiol., etc.*, Paris, 1858, tome ii. (1) p. 657, stated that sympathetic saliva was much more viscid than chorda saliva. The sympathetic secretion in the sheep and rabbit was noticed by v. Wittich, *Virchow's Archiv*, 1866, Bd. xxxvii. S. 93.

parotid of a rabbit 3 to 6 per cent. In the cat the percentage of organic substance in sympathetic saliva from the submaxillary gland is small (about 0·5 per cent.), and less than that in the chorda saliva.

The percentage of salts in sympathetic saliva does not exceed the percentage of the salts in saliva produced by stimulating the cranial nerve.

The analyses of the parotid saliva in the rabbit have been made by Heidenhain.[1] I extract the following from one experiment:—

Parotid Gland—Rabbit.

	Time of collecting Saliva.	Amount of Saliva.	Percentage of Organic Substance.	Percentage of Salts.
Saliva from stimulating *both* sympathetics . . .	38 min.	2·6 c.c.	4·93	0·54
Pilocarpine saliva from *one* gland	18 ,,	4·2 ,,	0·65	0·81

The sympathetic saliva in the cat[2] is, as I have said, usually less viscid than chorda saliva. But it is possible that a strong or prolonged stimulation of the sympathetic might give rise to a saliva with a higher percentage of solids than the chorda saliva. I append an analysis of sympathetic and chorda saliva in the cat, obtained by moderately strong interrupted currents.

	Percentage of Organic Substance.	Percentage of Salts.	Percentage of Solids.
Chorda saliva . . .	0·87	0·34	1·21
Sympathetic saliva . .	0·43	0·28	0·70

The sympathetic secretion in the cat is very much like the "augmented" secretion of the gland of the dog (cf. *infra*), in that it starts quickly, quickly becomes slow, and is watery. It differs in the rapidity of recovery from the effect of immediately preceding sympathetic stimulation. The maximum amount of secretion is obtained by stimulating fifteen seconds out of every thirty, or even for shorter periods.

In certain circumstances the sympathetic may produce a brief rapid secretion from any or all of the salivary glands. That is the case when it is stimulated shortly after stimulation of the cranial nerve. There is a rush of saliva, quickly following the sympathetic stimulation, reaching its maximum in a few seconds, and, after about seven to ten seconds, rapidly declining. A very brief stimulation of the cranial nerve is sufficient to increase in this way the amount of saliva obtained from the sympathetic. And thus, if the cranial nerve and the sympathetic nerve be stimulated alternately, a not inconsiderable quantity of

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 38.

[2] Langley, *Journ. Physiol.*, Cambridge and London, 1878, vol. i. p. 86; 1885, vol. vi. p. 92.

sympathetic saliva may be obtained. It is convenient to have some name for this unusually rapid sympathetic secretion, and I have called it the *augmented secretion.*[1]

In the dog, the saliva of the augmented secretion is, in its physical characters and apparently in its percentage composition, intermediate between sympathetic saliva and that obtained by stimulating the cranial nerve.

The augmented sympathetic saliva from the submaxillary gland of the dog is three to ten times as abundant as ordinary sympathetic saliva. In fifteen seconds about $\frac{1}{8}$ c.c. is usually secreted, but there may be as much at $\frac{1}{3}$ c.c. The amount of the augmented secretion from the parotid is one-third to one-half that of the submaxillary gland.

The augmenting effect of stimulating the cranial nerve disappears in time, although the sympathetic is not stimulated in the interval. In the submaxillary gland of the dog the greater part of the effect disappears in ten to fifteen minutes. In the parotid, it has usually completely disappeared in ten minutes. The rate of disappearance does not seem to be affected by the injection of atropine.

Mere vascular dilation does not cause an augmented secretion, for if atropine be given in sufficient quantity, to paralyse completely the cranial secretory nerves, stimulation of the cranial nerve, which still gives largely increased blood flow, does not increase to any considerable extent the sympathetic saliva obtained subsequently.

When the sympathetic nerve is stimulated two or three times in succession for rather short periods, say of thirty seconds, the augmenting effect of a preceding cranial nerve stimulation does not necessarily cease with the first stimulation, but is visible, though to a much less degree, in the second, and it may be in later stimulations. In the case of the dog's parotid the third stimulation usually gives no secretion at all.

The following extracts from the notes of experiments will illustrate the statements made above with regard to the augmented secretion:—

Submaxillary Gland—Dog—Stimulation of the Sympathetic after moderate Stimulation of the Chorda Tympani.

Saliva secreted in mm. during successive 30 secs., $35\frac{1}{2}$ mm. = ·25 c.c.	$1\frac{1}{2}$	$1\frac{1}{2}$	1	1	$53\frac{1}{2}$	20	1	20	4	2	1
Nerve stimulated	Sy.				Ch.			Sy.			

In the second sympathetic stimulation the flow of saliva was 16 mm. during the first fifteen seconds, and 4 mm. during the second fifteen seconds.

Submaxillary Gland—Dog—Stimulation of the Sympathetic after brief Stimulation of the Chorda Tympani.

Saliva flow every 30 secs. in mm.	0	5	1	0	15	1	0	0	0	$1\frac{1}{2}$	$\frac{1}{2}$
Nerve stimulated		Ch. (for 2 to 3 secs.)			Sy.					Sy.	

Longer stimulation of the chorda tympani has little effect upon the maximum rate of flow of the augmented secretion, but it leads apparently to a less rapid fall after the maximum is attained.

[1] *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 291.

Parotid Gland—Dog—Stimulation of the Sympathetic after Stimulation of Jacobson's Nerve.

Saliva flow every 30 secs. in mm.	0	35	14	1	3	0	0	0	31	76	{interval 11 minutes}	0	0	32	0	10	0
Nerve stimulated		J.	Sy.		Sy.		Sy.		J.	J.			Sy.	Sy.	J.		Sy.	

A brief rapid increase in the flow of saliva is obtained by stimulating the sympathetic during the action of pilocarpine and other alkaloids which cause a continuous free flow of saliva. After the first rapid rise the secretion becomes slower, and in the parotid gland of the dog stops altogether; in the submaxillary gland the secretion slowly continues.

Parotid Gland—Dog—Pilocarpine Injected.

Rise of saliva	6	6	35	7	3	0	0
			stim. symp.				

Effect of Protracted Stimulation on the Amount and Percentage Composition of Saliva.

During protracted stimulation, as was shown by Becker and Ludwig,[1] the percentage of solids in the saliva diminishes. They found a marked diminution in the percentage of organic substance; and generally, but not always, some diminution in the percentage of salts. The most striking experiment given by them is the following. The chorda tympani was stimulated, and successive portions of the saliva were analysed:—

	Amount of Saliva Collected.	Percentage of Organic Substance.	Percentage of Salts.	Percentage of Solids.
1st portion	10·6	1·19	0·79	1·98
2nd ,, ...	13·2	1·26	0·63	1·89
3rd ,, ...	14·4	0·62	0·54	1·16
4th ,, ...	13·9	0·27	0·48	0·75

The decrease in the percentage of salts is probably connected, as Heidenhain has pointed out, with the slower rate of secretion of saliva in the later portions collected.

Heidenhain[2] showed that the percentage of solids sinks during protracted secretion, not only in chorda saliva,[3] but also in sympathetic submaxillary saliva. In the following experiment the sympathetic was stimulated at short intervals during five and a half hours; the first and the last portions of saliva collected were analysed:—

	Time of Collection.	Amount Collected.	Percentage of Solids.
First portion . . .	80 minutes	0·68 grm.	3·73
Last ,, . . .	88 ,,	0·89 ,,	1·49

[1] *Ztschr. f. rat. Med.*, 1851, N. F., Bd. i. S. 278.

[2] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, S. 65.

[3] We have to refer so frequently to the saliva obtained from the submaxillary gland—(1) by stimulating the chorda tympani, (2) by stimulating the sympathetic, (3) by injecting pilocarpine—that we are driven to adopt the terms, chorda saliva, sympathetic saliva, and pilocarpine saliva, for the saliva obtained respectively in these circumstances.

He[1] showed, further, that in parotid saliva, obtained by stimulating Jacobson's nerve, there is similarly a decrease in the percentage of solids as the secretion goes on, and, no doubt, it is a general rule for all salivary glands.

Relation of the Rate of Secretion to the Percentage Composition of Saliva.

Heidenhain[2] investigated the relations existing between the rate of secretion and the percentage composition of saliva. He showed that an increase in the rate of secretion was accompanied by an increase in the percentage of salts, and this whether the gland had secreted for a long time or for a short time.

An example of this is given in the following experiment, in which the chorda tympani was stimulated with currents of varying strength, and a few c.c. of saliva collected in each case. The samples of saliva are arranged in the table in the order of their rate of secretion :—

Order of sample . .	5	1	7	3	8	4	2	9	6
Mean rate of secretion per min. in c.c. . .	·15	·18	·19	·22	1·6	2·0	2·2	2·5	3·2
Percentage of salts . .	·34	·29	·25	·32	·37	·58	·44	·57	·58

It will be noticed that the percentage of salts does not quite go hand in hand with the rate of secretion. But it is almost impossible to keep the rate of secretion constant during the time of collecting a sample of saliva, and to this the divergences may, in the main, be attributed.

A closer relation between the rate of flow and the percentage of saliva was observed by Werther,[3] and by Langley and Fletcher.[4] Heidenhain found the percentage of salts to have an upper limit, with increased rate of secretion. This he gave as ·5 to ·6 per cent., though in one case ·66 per cent. was found. Becher and Ludwig had earlier found in one case ·78 per cent. Werther, and Langley and Fletcher found the maximum percentage to be ·77. From the observations of the latter, it appears that the faster the rate of secretion, the less increase there is in the percentage of salts for a given increase in rate of secretion. This is indicated in the following table :—

Rate of Secretion per Minute in c.c.	Percentage of Salts.	Increase in Percentage of Salts, corresponding to an Increase of 0·1 c.c. per Minute in the Rate of Secretion.
·400	·472	·04
·500	·512	
·760	·599	·033
·900	·616	·012
1·333	·628	·003

[1] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 23. During eleven hours' stimulation the percentage of solids sank from 0·88 per cent. to 0·49 per cent.

[2] *Ibid.*, Bd. xvii. p. 1. Earlier observations on the same lines were given by him in 1868 in his "*Studien.*"

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 293.

[4] *Phil. Trans.*, London, 1889, p. 109.

The experiments on the parotid gland given by Heidenhain show a general but not a very close relation between the rate of secretion and the percentage of salts in the saliva.

Sodium chloride forms the larger part of the salts in saliva. The percentage both of this and of sodium carbonate varies directly with the rate of secretion.[1] The salts insoluble in water, the chief of which is calcium carbonate, do not seem to follow this rule, or at any rate only partially, for, whilst there is sometimes an increase in the percentage of insoluble salts, with increased rate of secretion, this is by no means always the case;[1] they appear to decrease in amount during the progress of the secretion, as if in part they arose from a store in the gland itself.

The following experiment from Werther will illustrate the variations in the percentage of different salts. The saliva was obtained from a dog by stimulating the chorda tympani :—

	Amount of Saliva obtained in c.c.	Rate of Secretion per Minute in c.c.	Percentage of Organic Substances.	Percentage of Salts.	Percentage of Insoluble Salts.	Percentage of NaCl.	Percentage of Na_2CO_3.
1	17·6	0·176	0·30	0·35	0·019	0·29	0·042
2	14·2	0·890	1·12	0·43	0·060	0·30	0·067
3	16·2	0·216	0·12	0·21	0·015	0·13	0·029
4	16·2	1·082	0·64	0·42	0·030	0·27	0·046

The relation thus determined between the percentage of salts and the rate of secretion, holds for chorda saliva and for pilocarpine saliva[2] secreted under normal conditions. But it is not a universal rule. Thus, sympathetic saliva has a much higher percentage of salts than corresponds to its rate of secretion, if chorda saliva be taken as a standard of comparison. And the rule does not hold for chorda or pilocarpine saliva, when the blood flow through the gland is much diminished, or when the character of the blood is much altered. On this I shall say more presently.

Heidenhain also showed that in a fresh gland an increase in the rate of secretion is accompanied by an increase in the percentage of organic substance in the saliva. In the experiment given below, for example, an increase in the rate of flow of the submaxillary saliva of the dog, from 0·14 c.c. to 0·87 c.c. in one minute, was accompanied by an increase from 0·52 to 1·54 in the percentage of organic substance. But when a certain amount of the stored-up substance of the gland cells has been secreted, an increase in rate of secretion no longer leads to an increase in the percentage of organic substance in the saliva secreted in a given time.

The closeness of the relation between percentage of organic substance and rate of secretion from a fresh gland seems to me to have been much exaggerated. No doubt there is a relation of the kind, but, in actual experiments, it is frequently overridden by other factors.

[1] Werther, *op. cit.*

[2] Langley and Fletcher, *op. cit., supra.*

Interval between each Period of Collection and the one before it.	Amount of Saliva in c.c.	Rate of Secretion in c.c. per Minute.	Percentage of Organic Substance.	Percentage of Salts.	Total Percentage of Solids.
...	3·5	0·14	0·52	0·22	0·74
2 minutes	3·5	0·87	1·54	0·56	2·10
3½ ,,	3·0	0·66	1·63	0·45	2·08
20 ,,	2·8	0·11	1·07	0·36	1·44
2 ,,	3·0	1·00	0·91	0·49	1·41
2 ,,	3·0	0·50	0·76	0·39	1·16
13 ,,	2·5	0·13	0·48	0·30	0·78
3 ,,	3·1	0·77	0·51	0·38	0·90
1 ,,	2·8	0·31	0·42	0·36	0·79

Some General Characters of Saliva, and its Microscopic Constituents.

The viscidity of saliva, secreted by mucous glands, is generally in proportion to the percentage of mucin which it contains. This, of course, would not be the case, if the amount of alkaline salt in the saliva increased in much larger proportion than the amount of mucin, for, with a given quantity of mucin, the viscidity of the fluid varies with the amount of the solvent.

Saliva, from albuminous or from mixed glands, may be either watery or thick, irrespective, within certain limits, of the percentage of organic substance present. Sublingual saliva and parotid saliva of the dog, when they have a high percentage of organic substance, have a tendency to turn into a jelly-like mass, and this may further separate into a clot and clear fluid.

In very watery saliva, freshly secreted, which has not been allowed to stand in the ducts, and which is examined without delay, nothing is to be seen under the microscope.

When saliva is allowed to stand a short time in the ducts, carbonic acid is given off from it, and, in consequence, calcium carbonate is precipitated; the precipitate renders the saliva cloudy, and under the microscope appears as very fine particles, or groups of particles. On irrigating such a specimen with dilute mineral acid, the particles are dissolved. The saliva also may contain leucocytes, and will certainly do so if it has been allowed to stay long in the gland ducts. In ordinary experimental conditions, leucocytes collect in the connective tissue of the glands, and migrate, at times in large numbers, into the ducts. The leucocytes at first show amœboid movement; later, they swell, become vacuolated, and form the bodies which have been called salivary corpuscles. The saliva may also contain some cells from the ducts which have been separated or injured by insertion of the cannula, some isolated nuclei, either of duct cells or of leucocytes, and occasionally a few small fat globules.

In viscid saliva of the submaxillary gland of the dog, spheres or clumps of secreted substance are present. The number and the character of these vary broadly with the viscidity of the freshly-secreted saliva, and are, so far as I have seen, independent of the way in which the secretion is brought about. As sympathetic saliva is usually much more viscid than chorda saliva, it usually contains these constituents in much larger number.[1]

The spheres vary in appearance. In the more viscid specimens of saliva they are pale, have a very faint outline, and appear homogeneous (pale spheres). As a rule they are 2 to 4 μ in diameter, but larger and smaller ones occur. In the less viscid specimens of saliva some spheres like these are also found, but most are more watery-looking and are still paler (very pale spheres); they vary much in size, but on an average are larger; they are apparently the swollen forms of the ordinary pale spheres. There are also, especially in more watery saliva, spheres which differ from the preceding in having a fairly sharp outline (vacuolar spheres). In the more viscid forms of saliva, clumps occur as well as the pale spheres, and they are more numerous the more viscid the saliva.

In saliva freshly secreted and freshly examined, the spheres and clumps may easily escape notice, even though they be present in hundreds in the field of the microscope. On standing they become more distinct, and they become obvious at the periphery of the drop, when they are still barely visible in the centre. In sufficiently viscid saliva the spheres and clumps are much distorted at the edge of the drop, and in still more viscid saliva most of them are drawn out into elongated masses.

Acetic acid, ·5 per cent. up to nearly glacial, makes the spheres and clumps very refractive and rather oily-looking. Glacial acetic acid causes them to swell up and become pale, and the clumps usually become vacuolated. Sodium hydrate causes them to swell up and disappear.

When saliva containing spheres and clumps is allowed to stand, these bodies slowly settle, forming, as they do so, masses often of considerable size. The addition of an equal volume of 5 to 20 per cent. sodium chloride allows them to sink much more rapidly; they make a white, slightly adherent, but not viscid layer at the bottom of the vessel.

On irrigating viscid saliva under a cover-slip, the fluid added mixes but slowly with the saliva, so that, instead of irrigating, it is sometimes better to mix a small drop of saliva with a small drop of the reagent, and to place a cover-slip on the mixture. Water causes the spheres and clumps to disappear, but up to a certain point they can again be made visible by acetic acid; 1 per cent. NaCl or Na_2CO_3 makes the outlines of the bodies more distinct; 1 per cent. osmic acid causes them to swell up and take a faint brown tint. Methylene-blue dissolved in Na_2CO_3, 1 per cent. stains them, but as a rule not very quickly. Picrocarmine, safranin, and other reagents stain them slowly. When saliva is mixed with one to two volumes of dilute neutral or alkaline salts, dilute or

[1] Eckhard, *Ztschr. f. rat. Med.*, 1866, Bd. xxviii. S. 120, found the sympathetic saliva from the parotid gland of the horse to be whitish and to contain fine particles. Schiff, "Leçons sur la digestion," p. 293, found the same with the first drops of saliva secreted reflexly after a pause. The characters described were no doubt due to a precipitation of calcium salts in the saliva contained within the ducts.

strong acids, the spheres and clumps gradually disappear. In strong solutions of neutral salts (*e.g.* 20 per cent. sodium chloride), they may be kept for months at any rate. Strong alcohol and mercuric chloride cause them to shrink and make them irregularly granular. Flemming's fluid turns many of them into vacuolated spheres, with sharp outline and a few distinct small granules. No mucous cells are seen in saliva after treatment with any of these reagents.

In the submaxillary saliva of the cat, vacuolar and pale spheres are found, but not the larger clumps.

Microscopical constituents in saliva have been described by Eckhard, Kühne, and Heidenhain. The account I have given above differs in several points from theirs.

The most obvious view to take of these microscopical constituents of saliva is, I think, that some of the mucous granules are turned bodily out of the alveolar cells,[1] the fluid passing through the cells being insufficient to dissolve them; and that by swelling up or massing together they make the various forms of spheres and clumps which are seen. But although the mucous granules behave with some reagents very much as do the small spheres of saliva, and have in some states very much the same appearance, their behaviour with acetic acid is strikingly different. The mucous granules, on treatment with dilute acetic acid, swell up and burst like bubbles;[2] the spheres in saliva, as we have seen, become refractive and obvious. Although it is possible that this difference may depend on differences in the surrounding fluids, it is sufficient to prevent more than a provisional acceptance of the view that the spheres of saliva are simply undissolved mucous granules.

Substances which are or which may be secreted in Saliva.[3]

In saliva obtained from mucous glands, the chief organic constituent is naturally mucin. Little is known with certainty of the varieties of mucin which exist. In mucous saliva, whilst most of the mucin is precipitated by acetic acid as a stringy lump, there is not infrequently a portion which is precipitated in fine particles, these making the fluid cloudy. A small quantity of proteid is also present, probably belonging to the class of globulins.

In saliva obtained from albuminous glands the proteid constituents are globulin (or a body allied to globulin), alkali albuminate, and a small amount of serum albumin.[4]

In typical mucous saliva, diastatic ferment is either absent, or present in mere traces; in saliva from albuminous glands, the amount of diastatic ferment is variable and independent of the percentage of proteid, but in the saliva of any one gland the diastatic action increases with the percentage of proteid present.

The salts are, so far as is known, the same in mucous and in albuminous saliva, although their percentage amount varies considerably in the saliva obtained from different glands. The bases found are sodium, potassium, calcium, and magnesium; the acids are hydrochloric acid, carbonic acid, phosphoric acid, and sulphuric acid. Sodium chloride is by far the largest constituent; after this comes usually

[1] Langley, *Proc. Roy. Soc. London*, 1886, vol. xi. p. 202.
[2] Langley, *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 433.
[3] See also article on "Composition of Saliva," p. 342.
[4] Kühne, "Lehrbuch. d. Physiol.," 1866.

sodium carbonate; calcium carbonate and calcium phosphate are kept in solution by the excess of carbonic acid, and precipitated as the gas escapes.

Saliva yields to a vacuum about twenty vols. per cent. of carbonic acid, and small quantities of oxygen and nitrogen;[1] the carbonic acid, however, is all or nearly all combined with sodium carbonate to form sodium bicarbonate.

In the saliva of man, potassium sulphocyanate is normally present.[2]

The alkalinity of saliva depends upon the presence of sodium carbonate. In man and in the dog the percentage of this salt varies from 0·08 to 0·19 per cent.

In disease,[2] traces of other substances have been found in the saliva of man, for example, urea and leucine. In diabetes, lactic acid has been found in the saliva; the presence of sugar has been denied by most observers, but affirmed by some. In jaundice, saliva does not usually contain either bile acids or bile pigments, but in some instances traces are said to occur. In cases of poisoning with salts of mercury, lead, and some other metals, small quantities of the salts may be present in saliva; it is stated, however, that the salts of arsenic are not secreted by the salivary glands.

An investigation into the character of the substances which can and which cannot be secreted by the salivary glands, would undoubtedly lead to interesting and valuable information. It is possible that, with bodies not acted on chemically, the size of the molecule is the determining factor. A beginning of such inquiry, though not from this point of view, was made by Bernard.[3] He experimented on the secretion from the submaxillary and parotid glands of the dog, and on the parotid glands of the horse. He found that potassium iodide was very readily secreted, whilst neither sugar,[4] ferrocyanide of potassium, nor lactate of iron was secreted by the salivary glands, though they were all secreted by the kidney. Iodide of iron, on the other hand, passed into the saliva.

When lithium citrate is injected into the blood, the spectrum of lithium can be detected in the first drops of saliva secreted.[5] And methylene-blue also passes into the saliva, but it does not appear to do so constantly. Sulphindigotate of soda, which is so readily secreted by the liver and kidney, is not secreted by the salivary glands;[6] after injecting large amounts into the blood, a small quantity may be found in the saliva, but there is no reason to believe that this is due to any cause other than diffusion.

Effects of the Cranial and Sympathetic Nerves on the Blood Flow.

The fundamental fact that the cranial nerve contains vaso-dilator fibres and the sympathetic vaso-constrictor fibres, has been already mentioned. If any salivary gland be exposed, it will be seen to flush on

[1] Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 686.

[2] Gamgee, "Physiological Chemistry," vol. ii., from whom much of this paragraph is taken.

[3] "Leçons de physiol. expérimentale," 1856, Bd. ii.

[4] On injecting a large quantity of dextrose into the blood, I have found sugar in the saliva, and in quantity which is, I think, much too large to be accounted for by diffusion.

[5] Langley and Fletcher, *Phil. Trans.*, London, 1889, vol. clxxx. p. 149.

[6] Eckhard, *Beitr. z. Physiol. C. Ludwig, z. s.* 70, *Geburtst.*, Leipzig, 1887, S. 13.

stimulating the cranial nerve, and to become pale on stimulating the sympathetic.

The more detailed examination[1] of the blood flow through the gland has been made almost exclusively on the submaxillary gland of the dog. The blood flowing ordinarily from the vein is dark; on stimulating the chorda tympani, the blood flow increases rapidly for ten to twenty seconds, and then slowly decreases to normal; the blood itself becomes arterial in colour. The degree of the increase naturally varies, the flow may be five times as fast as the normal. In favourable cases the vein pulsates, and when it is cut the blood issues in jets, somewhat as from a small artery. Bernard gives the normal blood flow through the gland as about 5 c.c. in a minute; and this has been approximately the rate of flow in my own experiments, in which anæsthetics were given. Von Frey found—presumably in very large dogs—the rate of blood flow through the gland to be much greater, about 12 c.c. in a minute. In v. Frey's experiments, stimulation of the chorda for ten seconds caused the rate of blood flow to be 3 to 7 c.c. in five seconds; the effect rapidly decreased on repeated stimulation; the flow was diminished by curari.

There are no complete observations on the changes in the gases of the blood as it passes through glands in rest and in activity, but some data are given by Bernard.[2]

According to Bidder,[3] the maximal blood pressure in the vein, as the result of stimulation of the chorda tympani, is 37 mm. of mercury.

On stimulating the sympathetic the blood becomes darker, and flows more and more slowly, the maximal effect being obtained in twenty to thirty seconds.

It is doubtful whether the sympathetic completely stops the blood flow in the normal submaxillary gland; it does so at times in an experiment, but this may be due to clotting occurring when the blood becomes slow. In the parotid the effect of the nerve appears to be greater.

The latent period of both chorda and sympathetic varies from a barely perceptible time to several seconds; it depends upon the strength of the stimulus, the number of previous stimulations, and other conditions; but, generally speaking, the latent period is longer with the chorda than with the sympathetic.

Both nerves have a rather long after-action. The maximal effect remains for ten to fifteen seconds, and the original rate of blood flow only recurs a minute or so after the end of the stimulation. The duration of the after-action depends, up to a certain limit, upon the duration of the stimulus; and it appears to be greater with the chorda tympani than with the sympathetic. These points, however, have not received much attention.

When both nerves are stimulated simultaneously with maximal currents, the sympathetic gets the upper hand during the stimulation,

[1] Bernard, *Journ. de l'anat. et physiol., etc.*, Paris, 1858, tome i. pp. 233, 649 (reprints from *Compt. rend. Acad. d. sc.*, Paris, of the same year); "Leçons sur les propriétés physiol., etc.," 1859; v. Frey, *Arb. a. d. physiol. Anst. zu Leipzig*, 1877, Bd. xi. S. 89; Langley, *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 316.

[2] Cf. "La chaleur animale," 1876, p. 179.

[3] *Arch. f. Anat. u. Physiol.*, Leipzig, 1866, S. 339.

and anæmia of the gland is produced as if the sympathetic alone were being stimulated. Von Frey, using brief stimuli—usually lasting about ten seconds—observed that the after-action was that of the chorda tympani, and in some cases the increase of blood flow after the stimulation appeared to be as great as if the chorda alone had been stimulated.

When, however, the sympathetic is stimulated with weak currents, and the chorda tympani with strong currents, there is, within certain limits, an algebraical summation of effects. And the constriction produced by a weak stimulation of the sympathetic may be more or less annulled by a strong stimulation of the chorda.

Mutual Effects of the Cranial and Sympathetic Nerves upon Secretion.

We have already mentioned, under the head of the augmented secretion (p. 496), the effect on the sympathetic saliva of a previous brief stimulation of the cerebral nerve.

When the chorda tympani and the sympathetic nerve in the cat are stimulated simultaneously with minimal currents of not too long duration, the amount of saliva obtained is greater than that which is obtained from either nerve alone.[1] In the dog the same effect may also be seen; at any rate, if the gland is in a state to allow the sympathetic to produce an augmented secretion.

As the currents are increased in strength, the amount of the saliva obtained by simultaneous stimulation becomes rapidly less and less in excess of that obtained by stimulating the chorda alone. And with a very moderate strength of sympathetic stimulation, the amount of saliva obtained by simultaneous stimulation falls below, and it may be very considerably below, that which is afforded by stimulation of the chorda by itself. The secretion is rapid for five or ten seconds, and then speedily becomes slow. The retarding effect of the sympathetic we may reasonably attribute to the diminution in the blood supply to the gland which it brings about.

In the parotid gland of the cat similar effects are seen on excitation of the sympathetic and of Jacobson's nerve. The sympathetic nerve in the dog has a very marked retarding action upon the flow of saliva produced by Jacobson's nerve from the parotid gland, and it may stop the flow altogether (cf. also p. 498).

Prolonged stimulation of the sympathetic reduces the irritability of the gland, so that, on subsequent stimulation of the chorda tympani, the saliva only appears after a long latent period, and but gradually acquires its normal rate of flow.

Czermak[2] was the first to call attention to the retarding action of the sympathetic upon the chorda secretion. He stated that in the dog, the sympathetic stopped the chorda secretion, and produced a condition of the gland of such nature that it did not for some time respond to stimulation of the chorda tympani. He referred the action to inhibitory fibres, which he believed to be present in the sympathetic. Eckhard[3] considered that the

[1] Langley, *Journ. Physiol.*, Cambridge and London, 1878, vol. i. p. 102.
[2] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1857, Bd. xxv. S. 3.
[3] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1860, Bd. ii. S. 95.

retarding effect of the sympathetic was due to the secretion produced by it being very thick and viscid, and in consequence blocking up the ducts. Heidenhain [1] attributed the action of the sympathetic to the lack of oxygen caused by the diminished blood supply.

The effects on the percentage composition of chorda saliva, caused by first obtaining a considerable quantity of sympathetic saliva, and *vice versâ*, were noted by Heidenhain.[2] He found that protracted stimulation of either nerve diminishes the percentage of organic substance in the secretion subsequently obtained by stimulating the other nerve.

(*a*) Thus stimulation of sympathetic for two hours—0·65 grm. of saliva secreted, containing 5·9 per cent. solids.

The chorda tympani was then stimulated for two hours.

Stimulation of sympathetic for about one and a quarter hours—0·54 grm. saliva, containing 2·4 per cent. of solids.

(*b*) Stimulation of the sympathetic for six hours reduced the percentage of the chorda saliva from 2·4 to 1·0.

Since the organic substance in the saliva comes in the main at any rate—entirely, so far as we know—from the substance stored up in the gland-cells, the facts given by Heidenhain show that the secretion obtained from the two nerves arises in part at least from the same gland-cells.

On microscopic examination of the submaxillary gland of the dog, after several hours' excitation, either of the chorda tympani or of the sympathetic, the alveoli are found to be changed to a very unequal degree, a few having still the ordinary resting characters. This renders it probable that the secreting fibres are not equally distributed to all the alveoli.

Since the sympathetic saliva contains a higher percentage of organic substance than chorda saliva, we should expect that simultaneous stimulation of the sympathetic and of the chorda tympani would give a saliva containing a less percentage of organic substance than sympathetic saliva, and a greater percentage than chorda saliva; and this is the case.

Heidenhain has shown that the chorda saliva which is obtained shortly after stimulating the sympathetic has a higher percentage of organic substance than that obtained before such stimulation. The saliva, however, soon becomes normal, usually after 2 to 3 c.c. have been secreted. The after-effect of sympathetic stimulation is comparable to the after-action caused by strong stimulation of the chorda tympani, of which we have already spoken (p. 505).

We have dealt chiefly with the submaxillary gland, but the mutual relations of the cranial and sympathetic nerves are essentially the same in other salivary glands, including the parotid of the dog, in which the sympathetic nerve by itself commonly gives no flow of saliva.

The following results, taken from experiments by Heidenhain, will serve to illustrate some points regarding the saliva secreted by the parotid gland when both the sympathetic and Jacobson's nerve are stimulated.

[1] Hermann's "Handbuch," Bd. v. S. 46.

[2] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, S. 71.

EXPERIMENT 1.—*The Parotid Gland of the Dog.*[1]

Saliva obtained by Stimulating—	Duration of Stimuli.	Amount of Saliva collected.	Percentage of Organic Substance.	Percentage of Salts.
Jacobson's nerve . .	18 min.	3·11 grms.	0·76	0·26
Jacobson's nerve and the sympathetic . .	30 min.	3·63 grms.	1·41	0·32

EXPERIMENT 2.—*The Parotid Gland of the Rabbit.*[2]

In this experiment pilocarpine was injected, and a sample of the saliva collected. The cervical sympathetic was then stimulated; during the stimulation the secretion became slower until it stopped; on its cessation the stimulation was also stopped. After a short time the flow began again; when about three drops had been secreted the sympathetic was again stimulated, and so on, till a second sample of saliva was collected.

Saliva obtained from	Rate of Secretion per Minute.	Percentage of Organic Substance.	Percentage of Salts.
Pilocarpine . . .	0·22 c.c.	0·39	0·85
Pilocarpine and sympathetic stimulation .	0·062 c.c.	3·62	0·75

EFFECT OF VARIATIONS IN THE AMOUNT AND QUALITY OF THE BLOOD SUPPLIED TO A GLAND, UPON THE AMOUNT AND PERCENTAGE COMPOSITION OF THE SALIVA SECRETED.

In order to form a satisfactory theory of the action of secretory nerves, it is of the greatest importance to know how far variations in the amount and character of the blood flowing through the gland affect the amount and character of the saliva. Our information on this point is unfortunately still vague in many respects.

Certain broad facts can be readily observed by compressing the carotid artery on one side, after tying the carotid artery on the other side, and the subclavian arteries on both. The gland-veins are cut, so that the amount of blood flowing through the gland can be roughly determined; and the chorda tympani is stimulated during different degrees of compression of the carotid.

When the carotid is compressed to a moderate extent, the chorda on stimulation will not cause so much increase in the blood flow through the gland as it otherwise would, but it will nevertheless cause a considerable increase, and the blood will issue from the vein of an arterial colour. In such case, according to Heidenhain,[3] the amount of saliva obtained by a given stimulus will be of normal amount.

[1] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 31.
[2] Heidenhain, *op. cit.*, S. 40.
[3] Heidenhain (*Stud. d. physiol. Inst. zu Breslau*, Leipzig, S. 98) appears to refer to an increase of blood flow above that occurring with partially compressed carotid, and not to an increase above the normal blood flow.

At a certain further stage of compression of the carotid, stimulation of the chorda will still cause an increase of blood flow from the gland, but the blood issuing from it, instead of being of an arterial colour, will be of a venous colour. In this case Heidenhain finds that the amount of saliva obtained by a given stimulus will be less than normal.

When the artery is so far compressed that little blood flows through the gland, and the chorda causes no increase in it, there is naturally a great decrease in the amount of saliva obtained by a given stimulus. If the stimulus last about a minute only, the decrease is in fact nearly as great as if the blood supply be entirely cut off. On allowing the blood to flow again through the gland, the chorda saliva does not at once attain its normal amount. Brief closure of the artery causes more or less protracted diminution in the efficiency of the chorda; it may be noted that the vaso-dilator effect of the chorda recovers more quickly than its secretory effect.

The following example, taken from Heidenhain,[1] may be given to illustrate some of the points mentioned above:—Dog, arteries to head tied, except left carotid. Wharton's duct connected with a tube graduated in millimetres. Gland-vein opened. The chorda tympani was stimulated for one minute, and the rise in millimetres of the saliva in the tube was noted each five seconds.

Saliva flow.—0, 20, 70, 50, 55, 45, 36, 30, 27, 29, 30, 28 = 410.

The artery was then clamped for five minutes; during the last minute the chorda was stimulated, the blood flow from the vein was very slight.

Saliva flow.—0, 0, 21, 32, 33, 17, 14, 8, 6, 7, 2, 2 = 142.

The carotid was left unclamped for eight minutes, then clamped for one minute, during which the chorda was stimulated.

Saliva flow.—0, 0, 0, 2, 4, 6, 5, 5, 5, 4, 5, 4 = 40.

The carotid was unclamped, but the stimulus kept up for two minutes. The blood flow from the vein was moderately increased. The saliva rose 69 and 51 mm.

The carotid remaining unclamped, the chorda was stimulated during the third minute. It caused a rise of saliva of 203 mm.

The effect of diminished blood supply upon the percentage composition of saliva has not been very fully investigated. But Eckhard[2] states that ligature of the veins of the submaxillary gland does not cause chorda saliva to alter its character and become like sympathetic saliva. And, according to Heidenhain,[3] diminution of the blood supply by compression of the carotid does not cause an appreciable increase in the percentage of solids in saliva.

Heidenhain's experiments undoubtedly show that, in certain circumstances, a diminution of the blood supply to the gland has no considerable influence upon the percentage of organic substance in the saliva, obtained by stimulating the cranial nerve. But this does not seem to me to hold in all circumstances, for, in some observations on the submaxillary gland of the dog, made by Fletcher and myself,[4] bleeding the animal, whilst decreasing the rate of the secretion of saliva produced by pilocarpine, largely increased the percentage of organic substance in the saliva.

[1] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, S. 93.
[2] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1860, Bd. ii. S. 212.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 33, 43.
[4] *Phil. Trans.*, London, 1889, vol. clxxx. p. 131.

There are no experiments which show definitely what is the effect on the percentage composition of saliva of a decrease of blood supply due to simple constriction of the vessels. When the cranial nerve is stimulated during compression of the carotid artery, the blood flowing through the gland flows through dilated vessels. When the diminution in blood supply is brought about by stimulating a vaso-constrictor nerve, the blood flowing through the gland flows through constricted vessels. It is probable that, in the former case, fluid passes more readily through the vessel walls; hence, with the same amount of organic substance secreted in the two cases, one saliva might have a low and the other a high percentage of organic substance.

Changes in the amount and character of the saliva[1] may, however, be produced by variations in the character of the blood. The injection of a considerable quantity of dilute salt solution, such as 0·2 per cent., leads to a considerable increase in the rate of secretion of saliva, whether this is set up by stimulating the chorda tympani or by injecting small quantities of pilocarpine. Up to a certain point the percentage of salts increases in the normal manner; beyond this the percentage ceases to increase and may fall. An increase in rate may also be produced by injecting into the blood 100 c.c. to 250 c.c. of stronger solution (as 2 per cent.) of sodium chloride or sodium carbonate. Probably this amount leads to the passage of water from the tissues, and so increases the volume of the blood. The injection may cause an increase in the percentage of salts. Injection of strong salt solution into the blood, in quantity sufficient to increase the percentage of sodium chloride in the serum, was found by Novi[2] to increase the percentage of the salt in submaxillary saliva; though never up to that in the serum. When a certain amount of strong salt solution (20 per cent.) is injected, the gland becomes œdematous, and neither placing acids on the tongue (Novi), nor stimulating the chorda tympani, nor injecting pilocarpine (Langley and Fletcher), will cause a secretion.

Relation of Secretion to the Flow of Lymph.

We know very little with regard to the flow of lymph from the glands in various conditions. The lymph vessels leave the submaxillary gland at the hilus. If the lymph could be collected and analysed, it would give information very much needed with regard to the secretory activity. Heidenhain,[3] who has paid some attention to the subject, appears only to have noticed whether œdema of the gland was produced or not, but it is manifest that if the lymph vessels were large there might be very great increase in the lymph flow without œdema.

Heidenhain[4] considers that there is no increase in lymph flow from the gland during stimulation of the chorda, either before or after giving atropine. Supposing, then, that atropine does not act on the vessel wall, so as to hinder the passage of fluid through it, it would follow that fluid passes from the vessels in increasing amount, as an increasing amount of saliva is secreted by the gland. In other words, it would follow that there is in rest a certain slight constant formation of lymph, and that,

[1] Cf. Langley and Fletcher, *op. cit.*
[2] *Arch. f. Anat. u. Physiol.*, Leipzig, 1888, Physiol. Abth., S. 403.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. ix. S. 346.
[4] Hermann's "Handbuch," 1880, Bd. i. Th. 1, S. 73.

when the gland secretes, an additional amount is formed exactly equal to that of the fluid in the saliva secreted,—a conclusion which it is not easy to accept.

In two conditions œdema of the gland is obtained: First, when dilute acid (0·5 per cent. HCl) or an alkaline salt (5 per cent. Na_2CO_3) is injected into the gland duct.[1] In this case, œdema is slowly produced; rapidly, however, if the chorda tympani be stimulated, though no secretion follows. There can be little hesitation in attributing this to the injury inflicted on the walls of the small vessels; for damage of the vessels, as we know, largely increases the amount of the lymph formed in any given condition. Secondly, when there is a considerable resistance to the flow of saliva from the duct. On continued stimulation of the chorda in such cases, the lobules become separated by a mucous fluid, and there is great œdema. At first this fluid consists simply of filtered saliva; later, probably, lymph is added, partly in consequence of a direct injury to the vessels, and partly, as suggested by Heidenhain, in consequence of pressure on the vein.

The Secretory Pressure.

Ludwig[2] was the first to show, by experiment on the submaxillary gland of the dog, that the secretory pressure may overpass considerably the blood pressure. Thus in one case he obtained a pressure of 190 mm. of mercury from the saliva caused to flow by stimulating the chordo-lingual nerve, although the blood pressure in the carotid artery was only 112 mm. of mercury. Since that time considerably higher pressures have been obtained from chorda saliva; the maximum pressure observable in any one species is, broadly speaking, the greater, the larger the individual.

On connecting Wharton's duct with a mercurial manometer, and stimulating the chorda tympani, the pressure rises at first rapidly, then more and more slowly; when the maximum pressure is attained, a cessation of the stimulus is followed by a fall of pressure, due to filtration taking place between the cells of the ducts and of the alveoli. When the observation is at all frequently repeated, the lobules of the gland become separated by mucous fluid, the pressure attained becomes less, and the irritability of the gland greatly decreases.

In the parotid gland of the dog, the observed secretory pressure is less than in the submaxillary gland, usually being 100 to 130 mm. of mercury, but the difference is probably due to the limpidity of the parotid saliva, which allows a more rapid filtration.

The pressure of the sympathetic secretion may also exceed that of arterial blood. In experimenting with a mercurial manometer, the pressure should be raised artificially to about 150 mm. of mercury during the first stimulation of the sympathetic, the connection of the manometer with Wharton's duct clamped for about thirty seconds, and then unclamped and the sympathetic again stimulated. Heidenhain,[3] in an experiment on the submaxillary gland of a dog, found that the sympathetic saliva was secreted at a pressure of 150 to 160 mm., whilst the pressure of the chorda saliva was 250 to 270 mm.

[1] Gianuzzi, *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, 1865.
[2] *Ztschr. f. rat. Med.*, 1851, N. F., Bd. i. S. 271.
[3] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, S. 69.

It may, however, be doubted whether there is such a difference in the maximum pressure. In the observations I have made on the point, stimulating alternately the chorda tympani and the sympathetic, the sympathetic has given a perceptible though slight and brief rise of pressure at approximately the maximum pressure obtainable from the chorda tympani.

Reflex Inhibition of the Salivary Secretion.

During the progress of secretion, a certain decrease in the rate of flow, or even a cessation, may be caused by stimulation of afferent nerves. Such an effect might be due—to select the most probable causes—either to an inhibition of the central secretory centre, or to a constriction of the blood vessels of the gland. The experiments have not, however, been directed to an accurate determination of the method of production of reflex inhibition.

Pawlow[1] states that the slow secretion induced by partial dyspnœa, or by curari, is decreased or temporarily stopped by stimulation of the sciatic for one or two minutes with a particular strength of current, or by exposure of the abdominal viscera. The experiments given can hardly be considered to be conclusive, and Buff[2] finds that, quite apart from stimulation, the secretion occurring in the conditions of Pawlow's experiments is not itself constant in rate.

Action of Alkaloids upon the Salivary Glands.

There are obviously a number of ways in which a substance introduced into the blood might cause a secretion of saliva. It might stimulate the peripheral endings of sensory nerves and produce a reflex secretion; it might stimulate some part of the central nervous system, the connections of the visceral nerve-fibres with the local nerve-cells, the nerve-cells directly, the nerve-endings in the gland, or finally the gland-cells directly. Of several of these modes of action we have no certain example. We shall confine our attention to those alkaloids, the effects of which have most served as a basis of physiological deduction.[3]

Atropine.—Atropine arrests the normal secretion from the glands of the mouth, nose, and pharnyx, so that the whole mucous membrane becomes dry. The arrest is due to a paralysis of the cranial secretory nerves, the strongest stimulation of them no longer causing a secretion.[4] In the dog, 10 to 15 mgrms. of atropine, when injected into a vein, produce the paralysis; in the cat, 3 to 5 mgrms. are sufficient. Considerably smaller doses than these reduce to very small limits the secretory power of the nerves; hence, in determining the minimal amount of atropine required to produce paralysis, it is advisable to stimulate the nerve for a minute or more, and to repeat this after a few minutes' interval.

The sympathetic nerve is either not paralysed at all, or only by a

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvi. S. 272 (experiments made on the submaxillary gland of the dog).

[2] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1888, Bd. xii. S. 3.

[3] A few only of the original papers dealing with this subject can be given here; fuller references will be found in treatises on pharmacology.

[4] Keuchel, "Das Atropin und die Hemmungsnerven," Dorpat, 1868; Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 309.

comparatively large dose of atropine.[1] In the dog more than 100 mgrms. may be injected into a vein, and still secretion will be obtained from the submaxillary gland by stimulating the cervical sympathetic. In the cat this nerve ceases to cause a secretion after about 30 mgrms. of atropine have been given.[2]

The point of action of atropine is the termination of the nerve-fibres around the gland-cells. There are several facts which show this. We may mention the following:—In the case of the submaxillary gland, when a dose of atropine has been given just sufficient to paralyse the chorda tympani, no secretion is obtained by stimulating peripherally of the (true) submaxillary ganglion; *i.e.*, the postganglionic nerve-fibres cause no secretion. Atropine applied directly to nerve-fibres—whether preganglionic or postganglionic—in their course towards a tissue, does not paralyse them. The paralysis produced by it must then be either one of nerve-endings or of gland-cells. But in the case we are considering the gland-cells are not paralysed, since they are at once set secreting by stimulating the cervical sympathetic. Hence we conclude that atropine acts upon and paralyses the nerve-endings of the postganglionic secretory fibres of the chorda tympani. And we may conclude, further, that in other cases in which atropine paralyses secretory nerves, it has this effect in consequence of an action upon the nerve-endings in the gland.

The exact method of action of atropine we can only guess at; we might suppose, either that it annuls the conductivity of the nerve-endings, or that it causes a retraction of the terminal filaments, in the manner suggested by Duval and others for the processes of nerve-cells in general, so that nervous impulses can no longer pass from the nerve-endings to the gland-cells.

Atropine does not paralyse the vaso-dilator fibres which accompany the cranial secretory nerves. This was first shown by Heidenhain[3] in the case of the chorda tympani of the dog. It is true that, when large doses of atropine are given, both vaso-dilator and vaso-constrictor glandular nerves produce less effect than normal, but there is nothing to show that this action is in any way specific.

Pilocarpine and muscarine.—Both pilocarpine and muscarine produce copious and prolonged secretion, when given in very small quantity; for example, when 1 or 2 mgrms. are injected into the blood.[4] The secretion when it slackens is increased by a further dose of the alkaloid, so that the flow of saliva can be kept up for a very long time, apparently indefinitely. A large dose is not required in order to produce the maximum rate of flow, its effect is rather to increase the duration of the flow. The saliva obtained is like that produced by stimulating the cerebral nerve, and the secretion is accompanied by a great dilation of the vessels of the gland.

The secretion to which these alkaloids give rise from the submaxillary gland is unaffected by section of the chorda tympani, or by extirpation of the superior cervical ganglion; it occurs after the connections of the chorda tympani with the local nerve-cells have been paralysed by

[1] Heidenhain, *op. cit.*

[2] Langley, *Journ. Physiol.*, Cambridge, 1878, vol. i. p. 98.

[3] *Op. cit.*, *supra.*

[4] The chief features of the action of muscarine were described, "Das Muscarin," Leipzig, by Schmiedeberg u. Koppe in 1869.

nicotine, and also after degeneration of the chorda tympani itself (cf. p. 519). The alkaloids therefore stimulate some peripheral structure. And as in the case of atropine, so with pilocarpine and muscarine, it is hardly open to doubt that the nerve-endings of the postganglionic fibres are the points of attack. The nerve-endings of the sympathetic nerve-fibres, on the other hand, are not stimulated by pilocarpine or by muscarine.

Stimulation of the chorda tympani during the pilocarpine secretion[1] produces in most circumstances an increase in the rate of flow, but when the secretion is as rapid, or nearly as rapid, as the alkaloid is capable of producing, the chorda has little or no effect. Further, after large doses of pilocarpine have been given, the chorda has also little or no effect; in the latter case, the apparent paralysing action may be due to the presence of more than one alkaloid in what passes for pilocarpine.

Stimulation of the sympathetic during the pilocarpine secretion causes a primary increase in rate, like that of the augmented secretion; after this there is a slowing, and if the stimulation be strong, there may be a complete cessation of the flow. The slowing effect is less in the submaxillary gland of the cat than in that of the dog, and less in the submaxillary of the dog than in the parotid of the dog.

The effect of the sympathetic in the last two cases is seen in the following extract from an experiment:[2]—

Dog.—*Pilocarpine Nitrate injected—Rise of Saliva in Tubes connected with the Ducts of the Submaxillary and Parotid Glands, taken every thirty seconds, in millimetres.*

Submaxillary .	25	54	9	3	2	1	0	0	0	1	3	11	19	29	32	32
Parotid . .	5	9	2	0	0	0	0	0	0	0	0	0	0	1	2	2
		stim. sympathetic														

The mutual antagonism[3] of atropine and pilocarpine (or muscarine).—If atropine, in quantity just sufficient to paralyse the chorda tympani, be injected into a vein of an animal, subsequent injection of pilocarpine or muscarine may or may not cause secretion. In many cases, as the amount of the alkaloid given is increased, death ensues, whilst the secretory nerves are still paralysed by atropine.

There are two methods by which the antagonistic action of two poisons on the salivary glands may be observed more satisfactorily than by injecting them both into the general circulation. The one is to inject the weaker poison in such a way that it passes through the vessels of the gland without entering the general circulation. The other method is to inject a small quantity of a rather strong solution of the weaker poison into the gland duct. In either case, the stronger poison is injected into the general circulation.

[1] Langley, *Journ. Anat. and Physiol.*, London, 1876, vol. xi. p. 173; *Journ. Physiol.*, Cambridge and London, 1878, vol. i. p. 339; Gley, *Arch. de physiol. norm. et path.*, Paris, 1889, p. 151.

[2] Langley, *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 826.

[3] For the action of physostigmine and its antagonistic action on atropine, cf. Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 309, and 1874, Bd. ix. S. 335. For the mutual antagonism of poisons in general, and especially as regards muscarine and atropine, cf. Prévost, *Arch. de physiol. norm. et path.*, Paris, 1877, p. 801.

I have tried both methods[1] in observations on the effects of pilocarpine and atropine upon the submaxillary gland of the cat and dog. The latter method is much simpler, and seems to me better. An experiment, briefly stated, is as follows. A paralysing dose of atropine is injected into a body vein. A cannula filled with a 2 to 4 per cent. solution of pilocarpine nitrate is tied into Wharton's duct, and 0·1 to 0·25 per cent. of the solution driven into the gland. This causes a secretion of saliva and great increase of blood flow, lasting several minutes, but steadily lessening in rate. During the flow of saliva the chorda tympani becomes again irritable, and may remain so for a short time after pilocarpine has ceased to produce a secretion. As the pilocarpine is carried out of the gland by the secretion, by the blood, and by the lymph, the atropine continually flowing to the gland in the blood again acquires the upper hand, and the nerve-endings become again paralysed. With renewed injection of pilocarpine there is renewed transient secretion and renewed transient irritability of the chorda tympani. And the paralysis and recovery may be repeated many times in an hour. It is, however, to be noticed, that if more than the minimal dose of atropine be given, more than one injection of pilocarpine may be required.

Although pilocarpine can instantaneously restore some degree of activity to the chorda tympani which has been paralysed by atropine, yet the activity is always considerably less than normal.

In the cat, when the cervical sympathetic has been paralysed by atropine, its activity can be restored by injecting pilocarpine into the duct, although pilocarpine does not stimulate the secretory nerve endings of the sympathetic.

Nicotine.—Nicotine causes a brief flow of saliva, followed by a temporary paralysis of the cranial and sympathetic fibres[2] up to their connections with the peripheral ganglia.[3] We have already described the main features of this paralysis in connection with the chorda tympani, and in connection with the sympathetic (p. 480). In all the mammals which have been experimented on, small doses of nicotine readily produce excitatory effects, but the amount required to paralyse the secretory and vasomotor preganglionic fibres varies widely in different cases. Moreover, the minimal amount required to produce paralysis is not precisely the same for fibres of different origin, or for fibres of similar origin but different function. In the rabbit and cat the differences are not great, the amount required varying from about 5 to about 10 mgrms. In these animals about 10 mgrms. of nicotine injected into the blood will cause a paralysis of preganglionic fibres lasting about fifteen minutes. In the dog, 30 to 40 mgrms. have a similar effect on the chorda tympani, in so far that, usually, stimulation of the chorda for about twenty seconds causes no secretion; but in some cases, at any rate, and even after larger doses, more protracted stimulation of the chorda induces gradually an active and protracted secretion,[4] continuing for some time after the cessation of the stimulus. And very large doses may be given to a dog without paralysing completely the cervical sympathetic.

[1] *Journ. Anat. and Physiol.*, London, 1876, vol. xi. p. 173 ; *Journ. Physiol.*, Cambridge and London, 1878, vol. i. p. 339 ; 1880, vol. iii. p. 2. For method of injecting into the gland arteries, cf. Heidenhain, *op. cit.*, 1874.

[2] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 316.

[3] Langley and Dickinson, *Proc. Roy. Soc. London*, 1889, vol. xvi. p. 423 ; Langley, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 123.

[4] Repeated doses have a tendency to cause in the dog a continuous secretion.

On the hypothesis that nicotine causes a contraction of the terminal fibrils of the chorda tympani, we might suppose that protracted stimulation leads to a slow gradual extension of the terminal fibrils, so that nervous impulses passing down the chorda tympani can again set up impulses in the peripheral nerve-cells.

Formation of Heat in the Submaxillary Gland.

The rapid flow of saliva caused by stimulating the chorda tympani suggested, not unnaturally, that a considerable formation of heat must take place in the submaxillary gland. Ludwig and Spiess,[1] using thermo-electric junctions, and Ludwig,[2] using thermometers specially designed, brought experimental proof that in the dog this was in fact the case.

Ludwig and Spiess placed one junction in the carotid artery, arranged as in the method of determining lateral blood pressure, so that the actual junction was, they said, in the full blood stream. The other junction was placed in a cannula connected with Wharton's duct, and apparently on the same side as that of the carotid taken. With a moderate rate of secretion they found the saliva to be about 1° C. warmer than the blood in the carotid.

Ludwig placed one thermometer in the carotid near its origin, and another in the course of a cannula connected with Wharton's duct of the opposite side. He states that there was in no case clotting in the carotid, but there does not seem to have been a flow of blood around the bulb of the thermometer. The room was kept at a temperature not less than 24° C. The saliva was found to be constantly of a higher temperature than the blood. The extent of this varied in different experiments, and, generally speaking, was greater the faster the secretion. The maximum difference found was 1°·6 C., the temperature of the saliva in this case being 41°·2 C., the rate of secretion 0·5 c.c. in 5·5 seconds. Ludwig gives also three experiments upon the respective temperatures of the blood in the carotid artery, of the blood issuing from the gland vein, and of the saliva. As a rule, the temperature of the venous blood was below that of the carotid blood, but occasionally it was slightly greater than that of carotid blood or of saliva. For example, in one case the temperature of the blood in the carotid was 39°·1 C., that of the saliva 39°·3 C., and that of the venous blood 39°·4 C.

The proof of an appreciable formation of heat during secretion appeared complete when Heidenhain [3] observed by the thermo-electric method that the temperature of the gland was often higher than that of the carotid blood, the difference in favour of the gland being still greater on stimulation of the sympathetic; and when Morat,[4] by the same method, obtained a rise of temperature in the submaxillary gland of the dog, on stimulating the sympathetic both after bleeding the animal to death and during temporary ligature of the carotid, subclavian, and vertebral arteries.

Bernard [5] plunged one thermo-electric junction needle in each gland, and found that stimulation of the chorda tympani caused a rise of temperature, and

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1857, Bd. xxv. S. 584; reprinted in *Ztschr. f. rat. Med.*, 1858, N. F., Bd. ii. S. 361.

[2] *Wien. med. Wchnschr.*, 1860, S. 433 and 449.

[3] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, Heft 4, S. 110.

[4] *Arch. de physiol. norm. et path.*, Paris, 1893, p. 285.

[5] "La chaleur animale," Paris, 1876, p. 325.

stimulation of the sympathetic caused a fall of temperature in the gland of the same side. He concluded that calorific nerve-fibres are present in the chorda tympani, and frigorific nerve-fibres in the sympathetic; but there is nothing in the account to show that the results were not due simply to a variation in the blood supply.

These results till recently passed unquestioned. But Bayliss and Hill,[1] on testing them, both by the thermo-electric and the thermometric methods, never found the chorda saliva to be warmer than the arterial blood. Their experiments differed in some points of method from Ludwig's. On one of these they consider the difference in result depends. The thermo-electric junction or the thermometer was pushed up the femoral artery into the aorta, so that it was exposed to the full current of blood. Bayliss and Hill consider that in Ludwig's experiment the temperature observed was less than the real temperature of arterial blood, so that, on stimulating the chorda tympani, the saliva secreted, though of a higher temperature than that recorded for the blood, was not of a higher temperature than that of the blood actually supplied to the gland.[2] And they came to the conclusion that no formation of heat in the submaxillary gland can be determined directly by any known method of measuring variations in temperature.

Supposing for a moment that this conclusion is correct, it does not of course mean that no heat is formed in the gland during secretion, but simply that the heat—undoubtedly set free by the chemical changes—is insufficient to cause an appreciable rise of temperature in the considerable mass made up of the saliva, the gland, and the blood flowing through the gland. But the main question can hardly be regarded as settled. For the tissues in the neighbourhood of the gland artery and of the duct are —at any rate, after placing a cannula in the duct and preparing the chorda tympani—at a lower temperature than the aortic blood. So that both the blood to the gland and the saliva secreted tend to become cooled. And thus it would be possible for the recorded temperature of the saliva to be less than that of aortic blood, although the temperature of the saliva secreted were higher than that of the blood supplied to the gland.

Electrical Changes in the Salivary Glands.

The electrical currents of the salivary glands of the dog and cat have been made the subject of observation by Bayliss and Bradford,[3] and by Bradford.[4] In such experiments, one non-polarisable electrode is placed upon the outer convex surface of the gland, and the other upon the gland close to the hilus. It is convenient to use Hermann's nomenclature for the currents which may be observed. When the outer surface of the gland is positive to the hilus, so that the direction of the current in the galvanometer circuit is towards the hilus, and in the

[1] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 351.

[2] It may be mentioned that the blood temperatures recorded by Bayliss and Hill are in nearly all cases less than those recorded by Ludwig, but no definite conclusion can be drawn from this.

[3] *Proc. Roy. Soc. London*, 1886, No. 243, p. 203; *Internat. Journ. Anat. and Histol.*, 1887, vol. iv. The ingoing current of the skin of the frog was discovered by du Bois Reymond in 1857. He attributed it to the glands present in the skin (cf. "Untersuch. ü. thierische Elektricität," 1860, Bd. ii.

[4] *Journ. Physiol.*, Cambridge and London, 1887, vol. viii. p. 86.

gland itself from the gland-cells to the surrounding tissue—the current is an ingoing current. When the outer surface of the gland is negative to the hilus, so that the direction of the current in the galvanometer circuit is from the hilus to the outer surface, and in the gland itself from the gland-cells towards the duct, the current is outgoing. The outgoing current, then, is one in the direction of the flow of the saliva secreted.

The current of rest may be either outgoing or ingoing. It is usually outgoing in the submaxillary gland of the dog, and usually ingoing in the submaxillary gland of the cat. The causes of the difference of direction have not been determined.

Any stimulation of nerves which causes a rapid flow of saliva will cause a strong outgoing current. When the flow of saliva is slight, the current, as a rule, is either diphasic, first outgoing and then ingoing, or ingoing only. Thus in the submaxillary or parotid gland of the dog, stimulation of the cranial nerve causes an outgoing current, and stimulation of the sympathetic, provided the secretion be slight, causes an ingoing current. The ingoing current begins less quickly and is less strong than the outgoing current.

In the submaxillary gland of the dog, the current of rest is said to vary from 1–500 to 1–10 of a volt. The outgoing current, caused by stimulating the chorda tympani, begins about 0·37 seconds after the beginning of the stimulation, and before saliva appears in the duct; it reaches its maximum before the maximum rate of secretion is attained. It may undergo temporary diminution or reversal, indicating the development of an ingoing current. The ingoing current, caused by stimulating the sympathetic, begins two to three seconds after the beginning of the stimulation, and only slowly attains its maximum.

In the submaxillary gland of the cat, stimulation, either of the chorda or of the sympathetic, causes, in most cases, first an outgoing and then an ingoing current.

Atropine annuls the effect of nerve stimulation, except perhaps in the case of the sympathetic of the dog; here the ingoing current produced by stimulation is much reduced, but it is not clear that it is completely abolished even by 100 mgrms. of atropine. Atropine annuls the outgoing current of stimulation before the ingoing. The amount of atropine required to abolish the outgoing current of stimulation is approximately that required to render the flow of saliva very slight. The amount of atropine required to abolish the ingoing current of stimulation is approximately that required to paralyse completely the secretory activity of the nerve stimulated (cf. p. 512).

Bradford attributes the ingoing current to "changes in the gland cells, leading to the elaboration of the organic constituents of the saliva," these being caused by the action of Heidenhain's trophic fibres, and thinks that the outgoing current is probably due "either to the passage of the fluid part of the secretion through the walls of the alveoli, or to the changes in the gland structures, that follow the excitation of a secretory nerve and precede the gland flow."

Most of the facts could be accounted for by supposing that the outgoing current is due to physical causes, namely, due to the passage of fluid through the gland-cells; and that the ingoing current is due to chemical causes, namely, the metabolic changes in the gland-cells, but the questions involved are too com-

plex to allow a definite conclusion to be arrived at. In any adequate discussion of the matter, the facts regarding the production of electric currents in other parts of the body, and especially in the skin and mucous membrane, would have to be taken into account. One or two points only we can mention here. In the skin and mucous membranes of the frog and other animals investigated, there is generally an ingoing electric current, which is increased by weak stimulation. Hermann[1] considers both currents to be due to an "apobiotic" change in the protoplasm. By "apobiotic" is meant any change which diminishes the vital energy of a part of the protoplasm, compared with the rest; such as is produced by stimulation, the act of dying, the change of protoplasm to mucin or to keratin, and so forth. Parts undergoing apobiotic change are negative to the rest of the protoplasm. Thus, in a mucous cell, the inner mucous portion of the cell becomes negative to the outer protoplasmic part, and a current is then set up, which passes in the galvanometer from capsule to hilus, and in the gland from mucous to protoplasmic portion, *i.e.* there is an ingoing current. As to the outgoing current, Hermann is inclined to consider it as a simple diminution (negative variation) of the normal ingoing or secretory current; whilst Biedermann advocates the view that the outgoing current is due to anabolic (assimilatory) processes in the gland-cells.

Section of Glandular Nerves. The Paralytic Secretion.

Claude Bernard[2] was the first to make observations upon the effect of section of glandular nerves. He found that section of the chorda tympani in the dog caused the submaxillary gland in two or three days to enter into a state of slow continuous secretion. The slow flow of saliva continued for five to six weeks, and then stopped. During this time the gland itself diminished more and more in size.

Since the secretion is the result of the section of nerve-fibres, it has been called the "paralytic secretion." Claude Bernard attributed the secretion to the complete removal of nervous impulses. Thus the flow of saliva did not begin for two or three days, because the terminations of the chorda tympani in the gland required two or three days to degenerate completely. It stopped in five to six weeks, because then, he thought, the chorda fibres had regenerated.

The question was taken up a few years later by Heidenhain.[3] In order to exclude the possibility of the paralytic secretion being caused by irritation of the duct or gland, he cut the chorda tympani in the tympanic cavity. The secretion occurred in the same way as when the nerve was cut peripherally of the ganglion, then called the submaxillary ganglion (cf. above, p. 481). It began in twenty-four hours at least, *i.e.* considerably earlier than the time given by Bernard. It was watery, and contained very little mucin; it contained many leucocytes (amœboide Körperchen), and was in consequence somewhat cloudy. The secretion was at first very slow, but gradually increased in rapidity, so that in about a week a large drop might be secreted every twenty minutes. After three weeks it diminished markedly. The gland itself, as its size diminished, became of a yellowish tint, and waxy appearance.

The time taken by the peripheral ends of the cut chorda tympani

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 246. References to much of the earlier work will be found in this paper.

[2] *Journ. de l'anat. et physiol., etc.*, Paris, 1864, tome i. p. 507.

[3] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, Heft 4, p. 73.

fibres to degenerate is not quite accurately known. Heidenhain states that in the dog, stimulation of the chorda causes a secretion three to four days after its section, and implies that later than this the nerve has no effect.[1] In an experiment on the cat, I[2] obtained a copious secretion by stimulating the cut end of the chorda three days after section; a secretion too copious, it seemed to me, to be attributed to the nerve-cells which sometimes occur in the region stimulated. But Bradford,[3] three days after section of the chordo-lingual nerve near the pterygoid muscle, obtained no secretion from stimulation of the nerve up to the point where the chorda tympani leaves the lingual. In the dog he found no effect five days after section, but no experiment was made at an earlier date. It appears, then, that the time required for a loss of irritability of the cut chorda tympani in the cat and dog lies somewhere between three and five days.

Notwithstanding the early loss of irritability of the chorda tympani after section, stimulation of its nerve-strands near the gland will in the cat still cause secretion. In this way I obtained a fairly rapid secretion thirteen days after section of the nerve, and a slight secretion in another experiment forty-two days after section of the nerve. And Bradford obtained secretion from the chorda tympani in the cat up to eleven days after section of the chordo-lingual. In his experiments he sometimes obtained a secretion by stimulating the chorda immediately after it had left the lingual nerve, but sometimes only when the electrodes were shifted farther towards the gland. In the dog, five or more days after section, he obtained no secretion by stimulating the chorda in any part of its course.

Vulpian[4] noticed in the dog, that a fortnight after section of the chorda tympani, injection of extract of jaborandi into a vein gave rise to a secretion, though less than normal. Extirpation of the superior cervical ganglion at the time of section did not affect the result. In the cat, I found that thirteen days after section of the chorda, venous injection of a few mgrms. of pilocarpine caused a copious secretion, and that forty-two days after section of the nerve, pilocarpine still caused a secretion, though distinctly less than on the opposite side.

These experiments, taken together with those already given on the action of nicotine (cf. p. 515), and with our general knowledge of the relation of visceral nerve-fibres to nerve-cells, show that, on section of the chorda tympani, its nerve-fibres degenerate in three to five days up to the peripheral nerve-cells. The nerve-cells are placed chiefly in the gland itself—more so in the dog than in the cat. And there can be little doubt that the variations observed as the result of stimulating the peripheral portions of the chorda depend in the main upon variations in the position of the peripheral ganglia. In some animals, postganglionic fibres are stimulated when the electrodes are placed on the strands outside the gland; in other animals, this only occurs when the electrodes are placed in the hilus. As the gland diminishes in size it naturally gives a less copious secretion under the influence of pilocarpine.

[1] Heidenhain (Hermann's "Handbuch," 1880, Bd. vi. S. 88) states that, although there was secretion, there was no increased flow of blood.

[2] *Journ. Physiol.*, Cambridge and London, 1885, vol. vi. p. 71.

[3] *Ibid.*, 1888, vol. ix. p. 304.

[4] *Compt. rend. Acad. d. sc.*, Paris, 1878, tome lxxxvii. p. 350. Before this, Prévost had stated that muscarine causes secretion after degeneration of the chorda tympani; cf. *Arch. de physiol. norm. et path.*, Paris, 1874, p. 719, note.

The peripheral nerve-cells[1] in connection with the gland may be spoken of as a local nerve-centre. This local centre is capable of exciting the gland-cells to activity long after the chorda tympani, which normally conveys impulses to it from the central nerve-centre, has degenerated.

Heidenhain suggested that the paralytic secretion might be due to a stimulation of the gland-cells by the decomposition products of the stagnating saliva. He observed that if the duct were clamped for about a day, a slow secretion of watery saliva ensued. The cases, however, are hardly comparable, inasmuch as, whilst the duct is closed, secretion is formed which partly distends the alveoli and partly is forced out of the ducts and lumina, and bathes all the tissues of the gland.

A final explanation can hardly yet be given, but some observations made on the cat lead me to think that the secretion is the result of nervous stimuli. In the cat the paralytic secretion is much diminished and even stopped by excess of chloroform and by apnœa; and is increased markedly by dyspnœa; the dyspnœic flow takes place more readily than on the opposite side, and, so far as can be judged, more readily than in a normal gland. These results indicate that the paralytic secretion is due chiefly, at any rate, to a slight continuous excitation of the local nerve mechanism.

Heidenhain found that the paralytic secretion also occurred in the dog, when the superior cervical ganglion was excised at the time of section of the chorda. In this case the secretion is due wholly to local changes. In the early stage of secretion in the cat, three days after section of the chorda alone, I noticed that section of the cervical sympathetic very much diminished or even stopped both the paralytic secretion and the dyspnœic secretion, although, in the later stages, section of the cervical sympathetic had little or no effect. Probably, then, if the sympathetic is intact, the secretion which occurs in the first few days after section of the chorda is largely due to impulses travelling down the sympathetic from the central nervous system.

The loss of weight which occurs in the submaxillary and the sublingual glands, after section of the chorda tympani, amounts in a few weeks to one-third to one-half of the original weight of the glands. Bradford has shown that section of Jacobson's nerve causes a similar loss of weight in the parotid gland[2] of the cat. Whether complete atrophy takes place, and if so what time it requires, there is no evidence to show.

In the submaxillary gland, and no doubt in the others also, the loss of weight is due to a loss of cell substance by the individual cells. And this loss is simply an instance of the gradual atrophy which occurs in tissues in the absence of functional activity. The persistent slight activity, of which the paralytic secretion is the sign, is quite insufficient to replace the normal exercise of function.

In the dog, according to Heidenhain, the paralytic gland contains a number of alveoli, presenting the appearance of the alveoli of an active gland. In my experiments, both on the dog and cat, the gland-cells were undoubtedly in the resting state. In the cat the saliva obtained by

[1] Six weeks after section of the chorda in the cat, when the submaxillary gland had lost one-third to one-half of its weight, the nerve-cells in the alcohol-hardened gland presented no certain difference from the nerve-cells of the gland of the opposite side.

[2] Bradford did not observe a paralytic secretion from the parotid.

stimulating the postganglionic chorda fibres, and by injecting pilocarpine, was distinctly viscid, and more viscid than normal. The alveoli mentioned by Heidenhain were, I am inclined to think, the demilunes which a decrease in the size of the mucous cells inevitably brings into prominence, notwithstanding an actual decrease in the size of the individual demilune cells.

Stimulation of the cervical sympathetic, during the progress of the paralytic secretion, has practically its normal action both in the dog and the cat. In the dog, it gives, when stimulated after a sufficient interval of rest, a brief quick flow of watery saliva, corresponding with the augmented secretion, and after this a slow slight secretion even thicker than usual. If the stimulus be prolonged, there is a long pause in the paralytic secretion, due partly to anæmia of the gland, and partly to the resistance offered to the flow by the thick saliva in the lumina and ducts. In the cat, the sympathetic produces secretion in the usual way and of the usual kind; and unless the stimulation be too prolonged, the paralytic secretion slowly creeps on in the intervals between the several stimulations.

Heidenhain noticed in the dog that section of the chorda tympani on one side caused a slight continuous secretion from the submaxillary gland of the opposite side. The occurrence of such a secretion I confirmed in the cat. It is convenient to have a name for this secretion, and I have called it the antiparalytic, or, more briefly, the antilytic secretion. In my experiments the antilytic secretion was stopped by apnœa and by excess of chloroform. Dyspnœa caused a secretion apparently greater than normal, though less than on the paralytic side. No certain antilytic secretion was observed either thirteen or forty-two days after section of the chorda. In its early stage, three days after section of the opposite chorda, it was diminished by cutting the chorda of the same side, and abolished by cutting the sympathetic also. So far, then, as regards the cat, there is some ground for thinking that the antilytic secretion is transitory and due to impulses set up in the central nervous system.

Section of the chorda tympani probably leads to slow changes in the nerve-cells of the secretory centre which are connected with the chorda fibres; these changes might make the central nerve-cells more irritable, so that they passed into a condition of continuous slight activity, thus producing the antilytic secretion. Or the antilytic secretion might, as suggested by Bradford, be simply a reflex from the tissues injured during the section of the chorda.

According to Heidenhain, the antilytic secretion in the dog continues after section of both chorda tympani and sympathetic nerves. As it is difficult to see why the local mechanism should be so easily thrown out of gear, it is best to wait for further observations on the matter.

Little is known as to the time taken for the chorda tympani fibres to regenerate. In a puppy, I obtained, three months after section of the chorda tympani, a secretion much as usual, on stimulating either the nerve which had been cut or the chordo-lingual, so that presumably regeneration is fairly complete in three months.

Section of the cervical sympathetic[1] has no observable permanent effect upon the gland, and it causes no paralytic secretion. The blood vessels for a time dilate, but this soon passes off. The nerve soon loses its

[1] Cf. Langley, *op. cit.*, and Bradford, *op. cit.*

irritability; in the cat, according to Bradford, it gives no secretion three days after section.

Stimulation of the ganglion will still cause secretion and pallor of the gland for several weeks after section of the nerve, and possibly indefinitely.

Excision of the superior cervical ganglion has also no certain effect upon the salivary glands, and does not give rise to a secretion. In the rabbit I could see no decrease in the size of the submaxillary glands, or alteration in their histological appearance, nine, sixteen, and twenty-three days respectively after removal of the ganglion, nor in a case in which the ganglion had been removed five years previously by Dr. Pye-Smith. The chorda tympani still causes secretion and flushing of the gland, though the flushing is apparently less than normal. Bradford removed the superior cervical ganglion in the cat. He found no atrophy of the gland up to seven weeks after the operation; indeed, in his cases both the submaxillary and the parotid glands were somewhat heavier on the operated than on the sound side. Removal of the ganglion causes the sympathetic filaments on the gland artery to degenerate, the loss of irritability being fairly rapid; thus, three days after the operation, Bradford obtained no secretion on stimulating these nerve-filaments.

Secretion due to a Reflex Action of Peripheral Ganglia.

We may reject the view of Bernard,[1] that a secretion can be obtained from the submaxillary gland of the dog, by means of nervous impulses passing from the mucous membrane of the tongue by the lingual nerve to the "submaxillary" ganglion, and thence to the gland. The direct proof alleged in favour of this view was that occasionally, after section of the chordo-lingual, direct stimulation of the tongue, or the application of ether, caused a slight secretion. As no anæsthetics were given, it is quite possible that a slight flow from the duct might be caused by reflex movements. The result was not obtained by Eckhard, Bidder, and others; and until it can be obtained with some constancy, and after administering at any rate a moderate amount of anæsthetics, it may properly be disregarded.

The indirect proof alleged is that after section of the chordo-lingual, stimulation of the lingual on its course to the tongue (the nerve being cut and the central end stimulated) causes a secretion from the submaxillary gland. This, in fact, is commonly the case. The amount of the secretion, broadly speaking, increases the nearer the electrodes are to the chorda tympani. It is often barely more than perceptible. The fact observed by Bernard, that three to five days after section of the chordo-lingual, a secretion could no longer be obtained, seems sufficient, with our present knowledge of the central nervous system, to show that the lingual secretion cannot be reflex in the ordinary sense.

There can be little doubt that Schiff's[2] explanation is in the main correct, namely, that some secretory fibres for the submaxillary gland, instead of running to it direct by the chorda tympani, accompany the lingual for a short distance and then run back to the gland. Schiff

[1] *Journ. de l'anat. et physiol., etc.*, Paris, 1864, tome i. p. 507. For some further account of the earlier papers, see Foster's "Text-book of Physiology," 1879, 3rd edition, p. 240.

[2] "Leçons sur la physiol. de la digestion," 1867, tome i. p. 284.

seems to have thought that these recurrent fibres ran in a single bundle a considerable distance down the lingual, for he says that when the lingual nerve is cut about 2 cm. beyond the point where the chorda tympani leaves it, and time is allowed for degeneration, no secretion is obtained by stimulating the central end; a negative result which was not obtained by Wertheimer.[1] Wertheimer's positive result then may be taken as showing that the recurrent fibres leave the lingual at more than one spot.

But it is nevertheless possible that on stimulating the central end of the lingual a secretion should be obtained which is not produced by recurrent fibres, and which is due to nervous impulses passing through local nerve-cells. The nerve-cells on the course of the chorda tympani are, as we have seen, scattered; if the chorda tympani fibres branch before running to these cells, stimulation of one of the branches would probably cause a nervous impulse to pass to the more central branches and to the cells connected with them. This would be a reflex through efferent fibres of the kind described in some other peripheral ganglia.[2] Such action with the actual anatomical arrangements is more likely to be obtained from the sublingual than from the submaxillary gland. It would, of course, be annulled by degeneration of the chorda tympani.

Direct Irritability of Gland-Cells.

It is natural to suppose that stimulation of the gland-cells by electrical, chemical, or mechanical stimuli should be capable of causing a secretion. There is, however, no direct evidence that this is the case. After atropine has been given, no secretion has been obtained; but it must be mentioned that, even when the nerve-endings in the submaxillary gland are in a full state of irritability, it is difficult to obtain secretion from it by electrical or other stimuli applied to its outer surface, and which do not affect the internal bundles of nerves.[3]

Extirpation of Salivary Glands, Injection of Saliva into the Blood.

The extirpation of all the salivary glands is, of course, impossible; but the large salivary glands, *i.e.* those which secrete by far the greater portion of the saliva, can be cut out. This has been done by Fehr.[4] He states that in the dog he removed not only the parotid, submaxillary, and sublingual glands on both sides, but also the orbital glands. The operation had no appreciable effect on nutrition; and the only difference in the behaviour of the animal was that it drank more water. A similar result was observed by Schäfer and Moore.[5] They removed from a dog the parotid, the submaxillary, and the larger part of the sublingual glands. There was no disturbance of nitrogenous metabolism, and neither sugar nor albumin appeared in the urine. Carbohydrates

[1] *Arch. de physiol. norm. et path.*, Paris, 1890, p. 519.

[2] Langley and Anderson, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 410.

[3] Bernard ("Leçons sur la propriétés physiologiques," etc., 1859, tome ii.) found, by stimulating the gland directly, that pain was caused.

[4] Henle and Meissner's *Jahresb.*, in *Ztschr. f. rat. Med.*, 1862, p. 255.

[5] "Proc. Physiol. Soc.," 1896 p. xiii., *Journ. Physiol.*, Cambridge and London, vol. xix.

were well digested, and the animal throve on a diet of bread and milk.

The salivary glands, then, in the domestic dog, appear to be rather a convenience than a necessity, and there is no evidence that they have any "internal secretion"; carbonic acid passes from the gland-cells to the blood, but there is no indication that any other substance does so.

Saliva injected into the blood is much less harmful than might be expected. Bernard,[1] indeed, injected considerable quantities into a vein of a dog to which no anæsthetics had been given, and did not observe a result of any kind. Extracts of the salivary glands injected into the blood cause a temporary fall of blood pressure,[2] but so many substances in solution do this that the action cannot be regarded as specific.

General Considerations; Theories as to the Mode of Action of Secretory Nerves.[3]

The facts which show that secretory nerve-fibres exist in the cranial nerves are so well known, that it is not necessary to consider them in detail. It is sufficient here to recall the fundamental facts, that secretion may in each salivary gland take place at a pressure higher than that of the blood supplied to the gland, and that nerve-fibres end in connection with the gland-cells.[4]

In the case of the sympathetic, the comparatively slight amount and the transitory nature of the secretion, render the question less clear. It was in fact suggested,[5] early in the history of sympathetic saliva, that the cervical sympathetic nerve causes a secretion solely in consequence of the pressure exercised on the gland-cells by the contraction of the blood vessels, brought about by stimulation of the nerve. Such a view offers a plausible explanation of many of the facts relating to the secretory action of the sympathetic, such as the normal small quantity of the secretion in the dog, the increased quantity after the cranial nerve has been stimulated, the rapidity with which the maximum rate of the "augmented" saliva is attained, the normal absence of reflex secretion by way of the sympathetic when sapid substances are placed on the tongue, and the absence of effect of atropine and pilocarpine upon the secretory function of the sympathetic.

But a closer inquiry shows, nevertheless, that this view is untenable. On the general theory it may be noted, that the constriction of the small arteries of the gland in all probability decreases the pressure on the gland-cells instead of increasing it. On the experimental side, we may mention three points.

1. The constriction of the blood vessels has at times no relation to

[1] "Leçons de physiol. expér.," 1856, p. 141.

[2] Schäfer and Oliver, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 277.

[3] For a general historical account of the views which have been held with regard to secretion, I may refer the reader to Prof. Gamgee's Address to the Biological Section of the British Association in 1882, and to Prof. Heidenhain's Introductory Account in Hermann's "Handbuch," 1880, Bd. v. Th. 1, S. 1–13.

[4] Cf. Fusari et Panasci, *Arch. ital. de biol.*, Turin, 1891, tome xiv.; G. Retzius, *Biol. Untersuch.*, Stockholm, 1892, N. F., Bde. iii., iv.; Korolkow, *Anat. Anz.*, Jena, 1892, Bd. vii. S. 580; A. Dogiel, *Arch. f. mikr., Anat.* Bonn, 1893, Bd. xlii.; Berkeley, *Johns Hopkins Hosp. Rep.*, Baltimore, 1894, vol. v.; C. Arnstein, *Anat. Anz.*, Jena, 1895, Bd. x. S. 410; G. C. Huber, *Journ. Exper. Med.*, Baltimore, 1896, vol. i. p. 281.

[5] Grünhagen, *Ztschr. f. rat. Med.*, 1868, Bd. xxxiii. S. 258.

the flow of saliva. Thus, on stimulating the cervical sympathetic in the dog, it may happen that the secretion does not begin until the pallor of the gland and the reduction of blood flow are about maximal; the slow flow of saliva may then continue without change in the blood flow, and may even continue after the end of the stimulation, when the blood vessels are dilated. In the cat, contraction of blood vessels without any flow of saliva can be easily observed by stimulating the sympathetic after about 30 mgrms. of atropine have been injected into the blood.

2. The quantity of saliva obtained by squeezing the gland is less than that obtained by stimulating the sympathetic. This is most readily observed in the submaxillary gland of the cat, in which about ten times as much saliva is usually obtained by stimulating the sympathetic as by squeezing the gland.

3. The total amount of saliva obtained by stimulating the sympathetic is, in some cases, too great for it to be obtained by simple expression of fluid from the gland. This is perhaps most striking in the case of the augmented secretion of the submaxillary gland of the dog. In favourable circumstances, $\frac{1}{5}$ to $\frac{1}{4}$ c.c. of saliva may be obtained by a single continuous stimulation, and with a diminution in the size of the gland not appreciably greater than would be accounted for by the diminution in the amount of blood in it.

Some of these observations, it will be observed, negative also the possibility that the sympathetic saliva can be due to pressure exercised by contractile tissue other than blood vessels around the alveoli.

We conclude, then, that both the cranial and the sympathetic nerves contain fibres which end in connection with the gland-cells, and which are capable of causing changes in the cells leading to secretion; and we pass on to consider whether the secretory nerve-fibres are of more than one kind. There are two possibilities to take into account:—first, whether there are fibres inhibiting the secretion as well as fibres exciting the secretion; and, secondly, whether there are fibres causing chemical changes in the gland distinct from those which cause the flow of fluid.

The former possibility we may treat briefly. Until it is shown that the decrease in the blood flow through the gland which the sympathetic causes is insufficient to account for the decrease in the flow of saliva which the sympathetic at times produces, this hypothesis of inhibitory fibres does not need serious attention.

The second possibility we must consider more at length. The theory of the existence of two kinds of nerve-fibres in secretory nerves is due to Heidenhain.[1]

According to this theory, the secretory fibres proper cause certain unknown changes in the cells leading to the passage of fluid through them. The trophic fibres cause chemical changes in the cells leading, on the one hand, to the growth of protoplasm, and, on the other, to the conversion of the stored-up secretory material into a more soluble form. Further, according to this theory, the proportion of these two kinds of nerve-fibres is different in cranial and sympathetic nerves. The cranial nerve contains more secretory than trophic fibres. The sympathetic nerve contains more trophic than secretory fibres.

The trophic fibres, it will be observed, have two functions, not necessarily connected with one another. The evidence that they cause

[1] Heidenhain, Hermann's "Handbuch," 1880, Bd. v. (1) p. 78.

a growth of protoplasm is derived from the microscopical examination of the various glands, after stimulating the sympathetic. Thus, according to Heidenhain—to take the most striking example adduced by him—if the cervical sympathetic be stimulated in the dog for several hours, there is no secretion from the parotid gland, but the gland-cells show a great increase in carmine-staining material, *i.e.* a considerable growth of protoplasm.

I have not been able to convince myself that any considerable changes of this nature take place. On stimulating the sympathetic the thick secretion usually stops up the ducts, and if any further secretion takes place it can only pass out into the lymph spaces. After stimulating the sympathetic for five to seven hours, I do not find any marked increase in the staining power of the cells; and the fresh gland either shows no outer non-granular zone at all, or a very small one.

The evidence that a separate class of trophic nerve-fibres exists, which converts stored-up material into a more soluble form, rests on certain facts, which we will discuss as far as possible separately. In the first place, there are the facts adduced to prove that soluble substance is formed during secretion, and which do not touch the question whether the formation is due to a special nerve-fibre or not.

1. It was shown by Heidenhain that the percentage of organic substance in saliva, secreted under the influence of the cranial nerve, increases with the rate of secretion. On this fact Heidenhain argued somewhat as follows: If the solvent power of the fluid passing through the cells remains constant, and the solubility of the stored-up substance in the cell also remains constant, the amount of the stored-up substance dissolved by the fluid in its passage through the cell will decrease as the rate of its passage increases. For, below saturation point, the amount dissolved must decrease the less the time the solvent is in contact with the solvend. But, in fact, the slower the passage of the solvent the less it dissolves; hence, with increasing rate of flow, there must be either an increase in the solvent power of the fluid, or an increase in the solubility of the stored-up substance. Heidenhain considered that in mucous saliva, at any rate, the only substance which could increase the solvent power of the fluid was sodium carbonate. And this salt, he found, did not increase, as saliva was secreted more rapidly. In consequence, he concluded that the substance in the cell must become more soluble. An increase in solubility of part of the stored-up substance was then a result of stimulating nerve-fibres.

But it is by no means clear that the rapidly-secreted fluid is not a better solvent than the fluid secreted slowly. Werther, working in Heidenhain's laboratory, found in fact that the percentage of sodium carbonate in the submaxillary saliva of the dog does increase, though but slightly, with the rate of secretion of saliva. And, in addition, it cannot be regarded as certain that sodium chloride and other neutral salts do not aid in the solution of the substances stored up in the cells. The evidence, indeed, seems to me to be on the other side. And, as we have seen, when saliva is secreted more rapidly, there is an increase in the percentage of salts as well as in that of organic substances. Finally, the statement that the faster the fluid passes through the cell, the less substance it will dissolve, depends on the assumption that in slowly-secreted and in rapidly-secreted saliva, the fluid has an equal opportunity of dissolving the stored-up material. This is not necessarily the

case; the more rapidly-flowing fluid might pass more freely into the intracellular spaces, and come into more intimate contact with the mucous or other stored-up material of the cell.

2. Better evidence of the formation of soluble substances in gland-cells, under the action of nerve stimulation, is afforded by the after-action of strong nerve stimulation. If, between two weak stimulations of a cranial nerve, a strong stimulation of the same nerve or a stimulation of the sympathetic nerve be introduced, the second weak nerve stimulation gives rise to saliva containing a higher percentage of organic substance than that produced by the first similar stimulation. The fact may be taken as showing that the strong stimulation, introduced between the two weak ones, has converted slightly soluble into more soluble material, which has only partially been carried out of the cell. But this is not the only possible explanation. We can imagine that the stronger the stimulus the more the fluid passing through the cell will be brought into contact with the stored-up substance, with the result that more of this substance will absorb water and pass a stage on the way to solution than would otherwise be the case. And consequently, for some time after a strong stimulus, any fluid passing through the cell would find substance already on the way to solution or already dissolved, without any alteration in its chemical composition.

The experimental evidence, then, of the formation of a soluble substance during secretion is not satisfactory. And, in fact, it is doubtful whether the glands contain any stored-up organic substance in a "comparatively insoluble" state. The granules of the glands are seen to enter readily into solution—micellar or other—when a crushed piece of the gland is irrigated with dilute alkaline salt solution. The mucin or mucins of saliva have not been shown to be different from the mucin or mucins contained in the salivary glands. The mucous material of the glands is often spoken of as mucigen, following the analogy of trypsinogen and pepsinogen; but it is well to remember that there is nothing to show that trypsinogen and pepsinogen are less soluble in dilute saline solution than trypsin and pepsin; and further, that there is some evidence that the œsophageal glands of the frog secrete pepsinogen as such, and not as pepsin.

Supposing, however, it were shown that nerve stimulation causes an increase in solubility of secretory material, it would still remain to show that this change is caused by a special class of nerve-fibres; and to this part of the theory we may now pass.

It was thought that direct proof of the separate existence of trophic fibres was afforded by the results on the parotid gland of stimulating the sympathetic in the dog. Stimulation of the sympathetic caused no flow of saliva, but caused nevertheless histological changes in the gland-cells, and a great increase in the percentage composition of the saliva obtained in other ways. Here was apparently an instance of nerve-fibres producing the changes demanded of the trophic fibres, by hypothesis, and producing no others.

But we have seen (p. 498) that the sympathetic is capable, in favourable circumstances, of causing a flow of saliva from the parotid gland of the dog. Since, then, secretory fibres are present in the sympathetic strand supplying the parotid, the action of the nerve in this particular instance cannot, without further examination, be taken to show the existence of an additional class of nerve-fibres.

We come, then, to a comparison of the relative effects of the cranial and sympathetic nerves as the final part of the evidence for the existence of two classes of nerve-fibres. It is said that the difference in the percentage composition of sympathetic saliva, and of that produced by stimulating the cranial nerve, can only be satisfactorily explained by supposing that secretory and trophic fibres are present in both, and that the number of trophic fibres relatively to the secretory is greater in the sympathetic than in the cranial nerves.

This conclusion seems to me to be legitimate and unavoidable, if a diminution in the blood supply to the glands brought about by vaso-constrictor nerves does not markedly increase the percentage of organic substance in the saliva secreted. But this, so far, has not been shown to be the case (cf. p. 508). The question can hardly be settled until means are found of stimulating the sympathetic vaso-constrictor fibres of the salivary glands without stimulating the sympathetic secretory fibres.

We find, then, that the hypothesis of a separate class of trophic fibres, although affording a convenient explanation of a certain number of facts, can hardly be considered proved at any point. It presents also certain difficulties of its own which we need not insist on here.

On the whole, I think the most probable view is, that only one kind of nerve-fibre runs to the gland-cells, and that this causes all the changes in the gland-cells which are capable of being caused by nerve stimulation. These changes include the taking up proteid material from the lymph, some katabolic action—shown by the setting free of carbonic acid—and changes leading to the passage of water and salts through the cell. It is not improbable that the nervous impulses hasten the conversion of absorbed proteid to secretory substances, and it is perhaps possible that they increase the solubility of the secretory substance already formed. The effect of the secretory fibres, as regards the amount and percentage composition of the saliva obtained, would naturally vary with the strength of the stimulus, the condition of the gland at the time, the quality and quantity of the blood flowing through the gland.

The exact processes which take place in gland-cells and which lead to secretion is at present outside the range of our knowledge. The high secretory pressure naturally suggests osmosis as the cause of the passage of water and of salts. And, about five and twenty years ago, the view that secretion is due to the formation in the cells of a substance of high endosmotic pressure was put forward by Hering and others. Much more is known now of the phenomena of osmosis than was known then; but the nature of the process is still so obscure, that to attempt to explain secretion on the lines of osmosis is to venture on little better than conjecture.

It may, however, be worth while to state briefly some points regarding the relation, or possible relation, of osmosis to secretion.

We will consider, first, what facts of secretion we could in some sort account for, on the theory that osmotic pressure is of the same nature as gaseous pressure, and assuming that osmosis does take place in the gland-cells.

The facts which it seems most feasible to offer an explanation of are, the occurrence of secretion when the cells are stimulated and not at other times, the increase in the rate of flow during stimulation, the increase in the percentage of salts in saliva with increase in the rate of flow.

We may speak of the alveolar cells as forming a membrane, and call the part towards the lymph the outer layer of it, and the part towards the lumina of the gland the inner layer. In the inner layer are spaces containing soluble organic substance.

The explanation of the above-mentioned facts on the osmotic theory might be as follows:—The membrane is impermeable in the unstimulated state; on stimulation, a rearrangement of its molecules takes place, so that, of immediately adjoining portions, parts are permeable to water and parts are permeable to salts also, whilst parts remain impermeable. On increasing the strength of the stimulation, a larger and larger area of the membrane becomes permeable, and of this a proportionately larger and larger part becomes permeable to salts.

The increase in the percentage of organic substance in saliva, which accompanies increased rate of flow, might be due simply to the greater percentage of salts causing an increase in the solvent power of the fluid, or to a larger proportion of the fluid passing into the spaces of the inner layer.

Proteid molecules do not pass through the gland-cells, but they enter it, and are deposited, forming the outer non-granular zone; the process is spoken of as the growth of protoplasm. We have reason to believe that the rate of growth of the protoplasm increases more than the rate of flow of fluid as the stimuli pass from weak to strong.

To account for this on the osmotic theory, it must be supposed that only the outermost portion of the membrane becomes permeable to proteids, so that the proteid molecules are blocked in their passage, and further that the ratio of permeability to proteids and to water is greater with strong than with weak stimuli. The theory becomes further complicated, if we have to apply it also to a taking up of proteid during rest (cf. p. 486), when there is no passage of fluid; for in this case the inner part of the membrane at least must be impermeable to water, whilst the outer part is permeable to proteids.

So far we have assumed that the conditions of the solutions on the two sides of the membrane are such as would lead to an osmotic flow through it, directed from its outer to its inner surface. But this is precisely the point it is difficult to be clear about. There is no obvious reason why the fluid in contact with surfaces of the membrane bounding the spaces should be very different from the fluid issuing from the inner surface of the membrane. But the saliva contains commonly less organic substance and less salts than the lymph. Why then should fluid pass from the lymph to the saliva? It can only be said that it is perhaps possible that a passage both of water and salts might take place if the organic substance in the spaces formed some combination with water and salts, of which at present we have not sufficient evidence.

The hypothesis which I have stated above seems capable of being put to the test of experiment, and of being either proved or disproved. Failing it, we are, I think, driven to suppose—apart from the hypothesis of special vital activity—that the outer layer of the cell forms a loose chemical combination with various substances of the lymph, and that these are passed on from molecule to molecule and disassociated at the inner surface. A process of this kind forms the basis of the chemical theory of osmosis. And it seems to me not improbable that such a process occurs in gland-cells, but it is extremely difficult to see how to bring any experimental evidence to bear directly on the question. The investigation appears to demand, as a preliminary, an intimate knowledge of the chemical nature of the membrane. The membrane consists of protoplasm. And there are few problems in physiology which appear more remote from solution than that of the chemical nature of living substance.

MECHANISM OF SECRETION OF GASTRIC, PANCREATIC, AND INTESTINAL JUICES.

By J. S. Edkins.

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The Mechanism of Gastric Secretion.

The histological appearances of the different secretory conditions of the stomach, and the relation of the secretory granules to the enzyme.—Though the existence of specific granules in secretory glands had previously been pointed out in connection with the pancreas and some salivary glands, it was not till 1879 that their existence was also observed in the secreting cells of the gastric mucous membrane by Langley and Sewall,[1] who showed that the chief or central cells are, in the resting condition, crowded with conspicuous granules, and that during digestion the granules in these cells diminish. As far as the ovoid or border cells are concerned, granules are to be seen in these, but they are much smaller in size, though quite discrete.

After digestion the cells take on different appearances, which consist mainly in the decrease of the number of granules. This decrease may be manifested in two different ways. In the first case, and the more typical, the outer border of the cell alone may show the lack of granules, the luminal border retaining them, unless in an extreme condition of exhaustion. In the second case, there may be a uniform decrease of granules throughout the cell, accompanied by a diminution in size of the cell, but unaccompanied by any formation of zones. These two forms of decrease may occur in different parts of the gastric mucous membrane of the same animal. Thus in the greater curvature of the stomach in both the rabbit and guinea-pig there is a formation of zones, in the cells of the fundus such a division is not seen.

[1] "Changes in Pepsin-forming Glands," *Journ. Physiol.*, Cambridge and London, 1879, vol. ii.

At the pyloric end of the dog's stomach in the resting or exhausted state, an appearance is seen which consists of small and obscure granules somewhat radially arranged, an appearance which bears a slight resemblance to that seen in the ducts of salivary glands in the fresh condition. A very marked difference exists, however, between the pyloric cells and the cardiac cells, and there seems little doubt that considerable histological distinction obtains. Nevertheless, attempts have been made by Ebstein [1] and others to prove the identity of the chief cells of the cardiac end with the lining cells of the body of the pyloric glands. It may be stated that Langley and Sewall found no difference in the pyloric cells whether the glands were in a resting or active condition, and other later observations also show a marked uniformity of appearance in the cells whatever the secretory condition be.

It may then be regarded as established that a diminution in the amount of granules characterises the chief cells as digestion advances. It has, moreover, been shown by Grützner [2] and others, that as digestion advances the fundus glands contain less ferment than in hunger. It is therefore justifiable to conclude that the granules are in some way connected with the ferment. In addition to this, we have the fact that more pepsin can be obtained from the cells of the fundus in the rabbit than from the greater curvature, and it is in the fundus that the cells are conspicuously granular. We have, however, to consider that, though the chief cells will yield pepsin, yet they do not actually contain pepsin. If the granules then are connected with pepsin, it must be in some antecedent form. The probable explanation of this is that the granules of the chief cells consist wholly or in part of *pepsinogen*, the precursor of pepsin.

The functions of the different forms of cells and of the different regions of the stomach.—Heidenhain originated the view that the chief cells were connected with the formation of pepsin, and the border cells with the formation of the acid of the gastric juice. The arguments upon which these conclusions are based are not direct, but though really inferential they appear to be supported by such evidence that but little doubt can be placed upon their accuracy.

The reasons for regarding the chief cells as connected with the formation of pepsin have been dwelt upon in the previous section. But is there evidence to disconnect the border cells from this same function? The most direct evidence we have is that, in the rabbit, the greater curvature contains more border cells than any other portion of the stomach; the pyloric glands of the smaller curvature contain at most an occasional border cell here and there, yet the amount of pepsin produced by the two gland forms is scarcely different. The obvious conclusion is that the border cells do not form the ferment. On the other hand, it is noticed that the pyloric secretion is distinctly alkaline if separated from the rest of the stomach; this is affirmed by Klemensiewicz,[3] Heidenhain,[4] and, later, Ackermann.[5] Apparently, therefore, such cells as are present

[1] *Arch. f. mikr. Anat.*, Bonn, 1870, Bd. vi.

[2] "Untersuch. ueber d. Bildung u. Aussch. des Pepsins," Breslau, 1875.

[3] "Ueber den Succus pylorieus," *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1875, Bd. lxxi.

[4] "Ueber die Pepsinbildung in den Pylorusdrüsen," *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii.

[5] "Experimentelle Beiträge zur Kenntniss des Pylorussecretes beim Hunde," *Skandin. Arch. f. Physiol.*, Leipzig, 1894, Bd. v.

in the pyloric region do not contribute to the formation of acid. In the frog the source of the ferment is an alkaline juice furnished by the œsophageal glands, whilst the cells in the stomach bear resemblance to the border cells of the mammal, and here alone the acid of the juice is secreted. The deeper parts of the cardiac glands, where there are fewer border cells, do not give an acid reaction, the acid reaction being evident only at the mouths and upper parts of the glands.

Claude Bernard[1] attempted to mark out the place where the free hydrochloric acid first appeared, by injecting intravenously a solution of ferric lactate followed by a solution of potassium ferrocyanide (these two compounds react with the production of Prussian blue only in the presence of a mineral acid). After the lapse of three quarters of an hour the animal was killed and the tissues examined. A blue precipitate was only observed on the surface of the mucous membrane of the stomach, especially in the neighbourhood of the lesser curvature, but no trace of blue in the glands. This experiment might, at first sight, be taken as indicating that the hydrochloric acid is first set free on the stomach itself, and is not formed in the cells of the gastric glands. Such a conclusion would be unwarranted. What the experiment does teach is that *there is no accumulation of acid in the cells*, but that the acid as rapidly as it is formed is thrown out of the cells as a secretion.

Brücke[2] tried to solve the same problem by exposing the stomach of an animal in which digestion was actively going on, and carefully removing all but the mucous coat; both in the pigeon and in the rabbit the reaction of the exposed mucous layer to litmus paper was found to be faintly alkaline or very faintly acid, practically neutral, but on testing the inner surface of the mucous membrane it was, as usual, intensely acid. This again is an experiment which, had it given a positive result, would have shown conclusively that the acid was secreted by the gland-cells; but, giving as it did a doubtful or negative result, it teaches little, and by no means proves the statement that the acid is not formed in the glands but in the stomach. In cutting into the stomach wall in this manner, sources of alkali are tapped in the small blood vessels and lymph spaces which are capable of supplying more than sufficient alkali to neutralise any acid in the gland lumina.

Brücke himself was not satisfied with this experiment, and attacked the problem by another method, which gave him results from which he concluded that the acid is really formed in the glands, and not in the stomach cavity. In birds, the gastric glands are compound glands forming flask-shaped bodies large enough to be easily seen without magnification. These compound glands possess also a flask-shaped cavity communicating with the stomach cavity by a comparatively narrow duct. Into this central cavity of the gland the secretion passes. Brücke took the secreting stomach of a fowl which had been killed during digestion, washed it out with magnesia suspended in water to neutralise the free acid on the surface of the mucous membrane, and sought out one of the above-described glands filled with secretion.

[1] "Leçons sur les propriétés physiol.," Paris, 1859, vol. ii.

[2] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1859, Bd. xxxvii.; "Vorlesungen," Aufl. 4, Bd. i. S. 306.

This he cut across, and tested the reaction of the fluid in its cavity. He found it as strongly acid as the secretion inside the stomach cavity. This experiment may be taken to show that the acid is secreted in the glands, but is continually being carried away by the stream of secretion. It might be objected against this experiment, that the fluid in the cavity of the compound gland is in communication with the stomach cavity and is acid by reason of admixture, but the communicating duct is too small to make this probable; moreover, there must be a continual stream flowing during secretion in the opposite direction from gland cavity to stomach cavity. That the gland cavity is not passively filled with secretion from the general stomach cavity, is also shown by the fact that some of these glands are swollen out with secretion while others are empty. It may be taken, then, that the gastric juice is acid as secreted by the gland-cells, and does not first become acid in the stomach.

Such observations as have been made in order to ascertain whether the border cells yield an acid reaction have not been successful. Though the mass of evidence is very greatly in favour of the view that the border cells are the origin of the acid of the juice, there are not wanting those who deny it entirely. Contejean [1] observed, as had previously been shown by Langley,[2] that the stomach cells of the frog, although they secrete acid, also secrete pepsin. But a more remarkable statement is that the pylorus cells secrete an acid juice. This is so much at variance with the results of the majority of investigations, that it cannot be accepted as correct. If it were true, a conclusive proof would be furnished against the view that the border cells originate the acid.

As regards the functions of the different regions of the stomach, it may be stated that the fundus and the greater curvature form in most animals pepsin, hydrochloric acid, and other constituents of the gastric juice. But considerable discussion has taken place as to the functional importance of the pyloric region. That an extract can be made from the pyloric region containing pepsin is generally agreed, but such an extract, in comparison with one prepared from the rest of the stomach, has very small digestive value. Langley,[2] in one experiment on the mucous membrane of the mole, found that if the digestive power of the pyloric region be taken as 1, that of the fundus would be 73. What then is the source of the pepsin that can be obtained from the pyloric mucous membrane? Is it pepsin formed by the gland cells in the pyloric region, or is it absorbed pepsin that has passed with the absorbed food into the mucous membrane of this region?

Wassmann [3] and v. Wittich [4] have held that the pepsin was merely infiltrated pepsin, and Wassmann stated that it was removable by repeated washing with water. On the other hand, Ebstein and Grützner [5] found that washing the mucous membrane of the pylorus causes but a very slow loss of pepsin. If, then, the cells of the mucous

[1] "Contribution a l'étude de la physiologie de l'estomac," *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892.

[2] *Proc. Roy. Soc. London*, 1881, No. 212.

[3] "De digestione nonnulla," Berolini, 1839.

[4] "Ueber die Pepsinwirkung der Pylorusdrüsen," *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii.

[5] "Ueber den Ort der Pepsinbildung in Magen," *ibid.*, 1872, Bd. vi.

membrane absorb and fix pepsin, they fix it in a somewhat stable combination. Such absorption would be comparable to that which, as v. Wittich pointed out, fibrin exerts when placed in a glycerin solution of pepsin, the fibrin rapidly absorbing the pepsin so as finally to render the glycerin solution inert. The pepsin so absorbed can only be recovered by treatment with hydrochloric acid and not by extraction with water. Finally, Klemensiewicz[1] and, later, Heidenhain[2] have isolated portions of the pyloric mucous membrane, and observed the secretion thereby obtained. But in Klemensiewicz's experiments the secretion was so mixed with abnormal fluids, such as pus, that the observations cannot be regarded as an index of the normal state of its composition. In Heidenhain's observations this difficulty was avoided by the adoption of antiseptic precautions. He obtained a secretion alkaline in reaction, viscous in character, rich in pepsin and rennet ferment. With hydrochloric acid 0·1 per cent. it digested fibrin very energetically, and caused milk to clot in about a quarter of an hour. Both Klemensiewicz and Heidenhain insist on the strong proteolytic powers of the secretion; the former even states that it digests fibrin as rapidly as the juice from the mucous membrane of the fundus. There is then a certain amount of disparity between the results of extracting the mucous membrane with various fluids, and testing the proteolytic powers of the extracts and those obtained by observing the peptic strength of the juice secreted by an isolated portion of the pyloric region of the stomach. We must therefore ask which furnishes us with the best criterion of the normal activity of the pyloric mucous membrane? By extracting with hydrochloric acid a large proportion of the pepsin present in the mucous membrane can ultimately be removed. But Klug[3] finds that, in order to obtain all the pepsin from the pyloric mucous membrane, it is necessary to make at least three successive extracts with hydrochloric acid, each one of the duration of twenty-four hours. The first extract shows little or no peptic activity; the second and third, marked activity. He ascribes the change of activity to the probability that the large amount of proteid present prevents the acid from separating the pepsin from its proteid compounds. But this difference is not found with the fundus, and it is somewhat difficult to understand why it should be more easy to extract the pepsin by acid from the fundus glands than the pyloric. We may regard it therefore as probably true that repeated extracts furnish us with a considerable amount of the pepsin obtainable. On the other hand, it may be asked how far the juices secreted into artificially isolated portions of pyloric mucous membrane are to be regarded as normal? It seems that in Heidenhain's case there was an absence of inflammatory conditions. But it must be noticed that the operation performed involved very considerable interference with the nerve supply to that portion of the mucous membrane. The mucous membrane was probably, therefore, to some degree in an abnormal condition. Nevertheless, the fact that proteolytic powers were shown could not be explained by reason of such an abnormal state; they must probably be indicative of normal secretion. It seems impossible that any "infiltrated" pepsin could produce the enduring effect in the secretion noticed by Heidenhain, and

[1] *Op. cit.* [2] *Op. cit.*

[3] "Untersuch. aus dem Gebiete der Magenverdauung," *Ungar. Arch. f. Med.*, Wiesbaden, 1894, Bd. iii.

it may be regarded as established that the pyloric glands do secrete pepsin.

The results of experiments that have been made to ascertain whether pepsin or pepsinogen is contained in saline extracts of the pyloric mucous membrane, lead to the conclusion that pepsinogen may be present. The fact that the amount of pepsin obtainable from the pyloric mucous membrane is increased rather than diminished[1] as digestion advances is susceptible of one of two explanations. It may be that during digestion the intracellular formation is more rapid than the secretion, and thus an absolute increase in the pepsin contents of the cells occurs. Or it may be that in course of absorption of certain products of digestion by the pyloric mucous membrane, a certain amount of pepsin becomes "infiltrated" in the membrane. At any rate, there is something fundamentally different in this respect between the secretion of pepsin at the pyloric end of the stomach and in the fundus glands, for in the latter the amount of pepsin decreases as digestion advances, whilst in the former there is an increase. Nor is there any evident change in the histological appearance of the pyloric cells corresponding to a secretory process. It is probable, as Langley has suggested, that the precursor of pepsin ("mesostate," as it may conveniently be called) is not so highly specialised as in the fundus glands; or there may be a series of mesostates, the more highly developed splitting off the enzyme earlier than the more lowly. The observations of Klug, which are confirmatory of the much older observations of Brücke, that a series of hydrochloric acid extracts will continue to show proteolytic powers, suggests that there are several mesostates of different grades coexistent in the cell. It is, moreover, possible that only in the most highly specialised mesostates does the condition of secretory granules obtain. From all this it appears probable that the formation of pepsin is a subsidiary function of the pyloric mucous membrane, and that it yet remains to be discovered whether the cells of the pyloric glands possess other more important functions.

The methods of obtaining gastric juice.—The older observers obtained samples of gastric juice by causing animals to swallow hollow perforated balls, containing pieces of sponge. In this way Réaumur,[2] Stevens,[3] and Spallanzani[4] obtained a fluid which caused meat to become digested, and which was marked by antiseptic properties. Tiedemann and Gmelin[5] made fasting animals swallow pebbles, which, acting as mechanical irritants, permitted a certain quantity of gastric juice to be secreted, and this was obtained by killing the animals shortly afterwards.

Beaumont had under observation in 1822 a man who had a gun-shot wound in the left side. There resulted from this a permanent fistula into the stomach. A valve, formed of the mucous membrane, became established over the opening, and on depressing this, introducing a tube, and turning the man on his left side, a flow of gastric juice was obtained.

[1] See Grützner's chart (Fig. 44), in the section on the variations in composition of gastric juice during digestion.

[2] "Sur la digestion," *Hist. Acad. roy. d. sc. de* (*Paris*), 1752.

[3] "De alimentorum concoctione," Edin., 1877.

[4] "Expériences sur la digestion de l'homme et de différentes espèces d'animaux," Genève, 1783.

[5] "Die Verdauung nach Versuchen," 1826.

Since this time many other cases of gastric fistula in the human subject have been under observation.[1] In 1842, Bassow[2] and Blondlot simultaneously introduced the method of obtaining gastric juice by an artificial fistula in an animal, and this method was further developed by Heidenhain,[3] who introduced antiseptic precautions into the operation.

Heidenhain also succeeded in so developing Klemensiewicz's method of isolating one portion of the stomach from the rest, that it was possible to keep the animals under protracted observation in this condition. In making the incisions for the operation, Heidenhain interfered as little as possible with the more important blood vessels, but he apparently produced some disturbance as far as the connections of the main nerves of the stomach were concerned.

Pawlow's[4] method of isolating by operation a portion of the stomach, retained the advantages of that of Heidenhain, while keeping unimpaired the nerve distribution to the isolated portion.

The influence of the nervous system on gastric secretion.—The stomach is supplied with two sets of nerve-fibres, cerebro-spinal and sympathetic. The vagi constitute the cerebro-spinal set, and branches from the solar plexus the sympathetic. The fibres of the vagi are almost entirely non-medullated in their course over the stomach. Plexuses formed by these nerves lie between the muscular and in the submucous coats. The nerve-fibres are distributed to the muscular tissue, to the blood vessels, and to the mucous membrane, and filaments have been traced to terminal arborisations between and in close contact with the cells of the gastric glands.[5]

Many have attempted to obtain indications of the nature of the impulses passing along these nerves by artificial stimulation.

Rutherford[6] cut the vagi during digestion, and found that the mucous membrane became paler. If the peripheral ends were stimulated, no regular effect resulted; if the central ends were stimulated, the mucous membrane became redder. After division of both vagi, apparently normal gastric juice was still secreted. Rutherford also found that normal secretion occurred after division of the splanchnics. The effect on the blood supply of stimulation of the central end of the vagus was, presumably, brought about by impulses passing to the medulla oblongata, inhibiting the action of the vasomotor centre there, and resulting in

[1] The following is a list of the principal observations on gastric fistulæ in man:—Helm, "Zwei Krankengeschichten," Wien, 1803; Brücke's "Vorlesungen," Bd. i. S. 300; Beaumont (1825–33), "Experiments and Observations on the Gastric Juice and the Physiology of Digestion," reprinted from the Plattsburgh edition, with notes by Andrew Combe, M.D., Edinburgh, 1838; W. Robertson, 1851; C. Schmidt, Diss., Dorpat, 1851; *Ann. d. Chem.*, 1854, Bd. xcii.; Kretschy, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1876, Bd. vi. S. 173; Uffelmann, *ibid.*, 1877, Bd. vii. S. 273; Richet, "Le suc gastrique," Paris, 1878.

[2] The following is a list of the more important observations on gastric fistulæ in animals:—Bassow, *Bull. Soc. imp. d. nat. de Moscou*, 1842, tome xvi.; Blondlot, "Traité analytique de la digestion," Nancy et Paris, 1843; Bardeleben, *Arch. f. physiol. Heilk.*, Stuttgart, 1849, Bd. viii.; Bidder u. Schmidt, "Die Verdauungssäfte," Leipzig, 1852; Holmgren, *Jahresb. ü. d. Leistung. . . . d. ges. Med.*, Berlin, 1860, Bd. i.; Schiff, "Leçons sur la physiologie de la digestion," Paris and Berlin, 1867, Bd. i. S. 15; Klemensiewicz, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1875, Bd. lxxi.; Panum, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1878, Bd. viii. S. 193; Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii. S. 169; 1878, Bd. xix. S. 148; Hermann's "Handbuch," Leipzig, 1881, Bd. v. S. 107.

[3] "Anlegung von Magenfisteln," Hermann's "Handbuch," Leipzig, 1881, Bd. v. Th. 1.

[4] The details of this method are described by Chischin, Inaug. Diss., St. Petersburg, 1894.

[5] Kytinanov, *Internat. Monatschr. f. Anat. u. Physiol.*, Leipzig, 1896, vol. xiii. p. 402.

[6] *Trans. Roy. Soc. Edin.*, 1870, vol. xxvi.

efferent impulses, dilator in character, passing along the sympathetic to the mucous membrane.

That impulses passing along the vagi influence the movement of the stomach, and possibly by that means to some extent the secretion, has been shown by several observers.

Goltz[1] exposed the stomach and œsophagus of two curarised frogs, and, after suspending them, dropped into their mouths salt solution. One, however, had had the brain and spinal cord destroyed. After a time it was found that in the complete animal the stomach and œsophagus were widely distended, whilst in the pithed frog they were empty. The latter result occurred equally well if the vagi only were cut. Stimulation of the vagi peripherally caused only slight contractions. The explanation given by Goltz of this result is that the stomach walls presumably contain a local mechanism which, under the conditions in which the animals were, would have undergone stimulation. The result of this would have been peristaltic contraction, causing the fluid to be passed on into the intestine. But ordinarily this is controlled by efferent impulses passing from higher centres along the vagi. If the controlling influence is destroyed, there results an exaggerated action of these centres. Goltz was disposed to regard this local mechanism as of a ganglionic nature.

In connection with this question, it may be stated that Openchowski[2] has described the existence of special nerve-plexuses with ganglionic cells, both at the cardia and at the pylorus. He considers that the opening and closing of these passages are to be referred to the direct influence of these ganglia, though these again are under the control of the central nervous system. Openchowski describes a centre for the contraction of the cardia situated in the posterior corpora quadrigemina, and connected with the stomach mainly by the vagi. A centre for the opening of the cardiac orifice lies in the basal ganglia, and communicates with the stomach by means of the vagus. There are also subsidiary centres in the spinal cord influencing dilatation of the cardiac orifice. In the same regions are centres influencing movements of the pylorus and the intermediate walls of the stomach. Openchowski emphasises the antagonism between the movements of the cardia and the pylorus; such nervous impulses as proceed down the vagus and dilate the cardiac orifice simultaneously close the pylorus.

As regards the more direct influence of impulses proceeding along the vagi upon secretion in the stomach, for a long time the greatest uncertainty prevailed, and it was held that in general such impulses did not directly affect secretion, but merely indirectly, through promoting movements of the stomach walls. Heidenhain[3] has suggested that, as mechanical irritation produced secretion from the digestive glands in the plant *Drosera*, so the direct irritation of food in the stomach might stimulate the gastric epithelium. There has existed for a long time, however, indirect evidence of a flow of gastric juice resulting from psychical conditions. Bidder and Schmidt[4] noticed, as early as 1852, that the sight of food in a gastrostomised dog resulted in an abnormal flow of gastric juice. To obviate any possibility of swallowed saliva causing this result, which saliva it was known could be secreted as the con-

[1] "Studien über die Bewegung von Speiseröhre und Magen d. Frosche," *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi.

[2] "Ueber die nervösen Vorrichtungen des Magens," *Centralbl. f. Physiol.*, Leipzig u. Wien, 1889, Bd. iii.

[3] Hermann's "Handbuch," Leipzig, 1881, Bd. v. Th. 1.

[4] "Die Verdauungssäfte und der Stoffwechsel," Leipzig, 1852.

sequence of psychical conditions, œsophageal fistulæ were made, and the saliva was prevented from passing into the stomach.

In Richet's[1] observations on a human subject, who, by swallowing a caustic alkali, had rendered the œsophagus impassable, and in whom consequently it had become necessary to make an opening into the stomach, it was observed that chewing savoury food, none of which passed into the stomach, produced a copious flow of gastric juice. It was not possible in these cases to absolutely assert that this resulted from nervous influence acting directly on the secreting epithelium, for movements of the stomach may have brought about the flow, but the quantity secreted suggested a direct nervous influence. That there is such a direct nervous influence has been conclusively proved by Pawlow,[2] in conjunction with Schoumow-Simanowsky. Their experiments were made on dogs which had had a portion of the stomach isolated in the manner already described, care being taken that the nervous connections were intact. In addition, the œsophagus was separated, the cut ends being attached to openings in the neck, so that swallowed food passed out at one opening, and, through the other, food which it was desired should enter the stomach could be passed in. It was possible, therefore, to bring the animal under the influence of food in three ways. In the first place, it might be shown food, but the food would not be actually introduced into the stomach. This constituted the so-called "psychical feeding."[3] Secondly, the animal might be fed by the mouth, but none of the food allowed to enter the stomach, since it would make its exit at the œsophageal opening. This was described as "pseudo-feeding" (Scheinfütterung). Thirdly, by the introduction of food into the lower division of the œsophagus, true feeding was carried on. The results varied according to the method adopted. The latent period, or period elapsing after administration before secretion commences, is in the dog about seven minutes. This does not vary much whether it be a case of psychical, pseudo-, or true feeding. The latter course of secretion shows, however, considerable variations. This was the first point established by Pawlow.

He next attempted to discover the paths of the nervous impulses bringing about these changes. Section of the splanchnics did not affect the results, but after severing both vagi the reflex secretion was absent. With one vagus cut (the right), the animal was found to respond in the usual way. Later the left vagus was divided without anæsthesia. The animals live for a few days in this condition, but during this time no reflex secretion occurs. From this it was concluded that the impulses constituting the efferent portion of the reflex act pass along the vagi. But more positive results in this connection were obtained by Pawlow by stimulating the peripheral ends of the cut vagi. If, some twenty-four hours after the second section, the cut end be stimulated,—and it is better to do this by applying induction shocks at the rate of one per second, rather than by using the rapidly interrupted

[1] "Le suc gastrique chez l'homme et les animaux," Paris, 1878.

[2] "Die Innervation der Magendrüsen beim Hunde," *Arch. f. Physiol.*, Leipzig, 1895.

[3] Though the exhibition of food to the dog, which had been operated on in the manner described above, evoked a flow of gastric juice, if, from previous experience, the dog was led to understand that the food would not actually be given, the secretion very soon stopped. It may be mentioned that Heidenhain did not regularly obtain a flow as the result of showing the animal food, and this suggests that his method involved interference with the nervous tracts.

current,—there results a flow of gastric juice. A certain interval occurs before the secretion is manifest, which is to be referred to changes actually taking place in the epithelium of the stomach. The character of the secretion varies according to the stage of the digestive act and the nature of the food. This will be again referred to in a later section.

It must be mentioned that some observers have not had the same success in showing the secretory importance of the vagus nerve. That certain differences are perceptible in consequence of its excitation, they admit, but they refer the variations rather to the altered power of muscular contraction possessed by the stomach. Thus Leubuscher and Schäfer[1] experimented both upon rabbits and dogs, performing the operations according to Pawlow's method. They frequently used a control animal for precision in estimating the change. As far as variations in the amount of hydrochloric acid were concerned, they came to the conclusion that there was no constant difference. The stomach they found invariably relaxed, and the food introduced within was, after a prolonged interval, found to be arranged in two zones, one against the walls of the stomach, which was well digested and contained hydrochloric acid in normal amount, that occupying the centre of the viscus being poor in acid and rapidly becoming decomposed. As far as stimulation of the peripheral stump of the vagus was concerned, they did not obtain constant results, but they appear to have adopted the rapidly interrupted current rather than the induction shocks at prolonged intervals used by Pawlow. That the food was not perfectly mixed, is obvious from their experiments. This will probably explain the decomposition occurring; this change was noticed also by Pawlow to come on a few days after section of both vagi. It is, however, to be noted that they were unable to confirm the passage of secretory impulses along the vagus.

There exists one observation upon the human being from which it seems that stimulation of the vagus may cause a flow of gastric juice. Regnard and Loye[2] stimulated the vagus in a decapitated criminal some forty-five minutes after death. Numerous drops of gastric juice appeared over the surface of the stomach. This may also, however, have been the result of movements of the stomach, which they observed at the same time to occur.

The conditions under which local stimulation provokes secretion.—As already stated, it has been held by many that simple mechanical irritation of the mucous membrane will directly produce a flow of gastric juice. Beaumont[3] showed that mechanical irritation of the mucous membrane caused increased vascularity and the appearance of small drops of gastric juice, and he further pointed out that the effect is confined to the irritated locality, and that the amount of juice secreted is small in quantity. Tiedemann and Gmelin,[4] Heidenhain,[5] and others also state that the secretion is limited. Pawlow[6] states that the amount secreted is practically nothing, when indigestible substances such as pebbles are placed in the stomach. It may therefore be regarded as probably true that any secretion produced by simple mechanical irritation is extremely small, and the existence of this slight secretion in no way suggests that the normal secretion can be looked upon as the result, to any great extent, of such stimulation. On the other hand, stimulation by food, even if solid, is much more effectual.

[1] "Ueber die Beziehungen des Nervus Vagus zur Salzsäure-secretion des Magenschleimhaut," *Centralbl. f. innere Med.*, Leipzig, 1894, Bd. xv.

[2] "Recherches faites à Amiens sur les restes d'un supplicié," *Compt. rend. Soc. de biol.*, Paris, 1887.

[3] *Op. cit.* [4] *Op. cit.* [5] *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix.

[6] Address at St. Petersburg. Reported in *Brit. Med. Journ.*, London, 1895.

Heidenhain found that following a latent period of some fifteen minutes after placing food in the organ, the stomach commenced to secrete gastric juice. This delay beyond the interval observed in Pawlow's experiments was presumably due to a certain amount of injury to the nervous connections. If indigestible substances were swallowed, the secretion was much longer delayed. The conclusion which Heidenhain arrived at was that certain products of digestion when absorbed stimulate the flow of gastric juice. The question then arises, What are these products of digestion, and by what paths are they absorbed? Are the completely digested foodstuffs (that is to say, completely digested as far as gastric digestion is concerned) passed on to the intestine and there absorbed, or are they directly absorbed in the stomach?

As regards the change undergone by different proteids when subject to gastric digestion, there is reason to believe that the stage reached in the stomach is not a final one, some further change taking place in the duodenum, and that the amount of peptone formed in the stomach may not be large, the proteose stage being, to a great extent, the final stage of gastric digestion. If this is so, and if the secretion from the gastric mucous membrane is influenced by absorbed peptones, it must be influenced by peptones absorbed in the small intestine. On the other hand, we are unable to state definitely to what extent the intermediate results of the digestion of proteids are absorbed in the stomach. As regards the carbohydrate foodstuffs, v. Mering[1] has shown that sugars are absorbed by the stomach. If it is absorbed digestive products that provoke the secretion, is it a specific product or products that cause this to occur, or is it a common characteristic of all? Chischin[2] has attempted to answer this question. He finds that feeding a dog (which has had a portion of its stomach isolated after the manner of Pawlow) with different varieties of food, results in very different characters being shown by the secreted juice during the course of digestion, and he hence infers that there must be some specific stimulus or stimuli influencing the secretion. The different substances were administered in such a manner as to avoid the "psychical" influence on the secretion. The administration of distilled water, gastric juice, or simple hydrochloric acid, caused but little change. Egg-albumin, sugar and starch solution, were tested with the same negative results. The administration of peptone, however, resulted in a pronounced secretion. Chischin considers that peptone was not only able to cause the gastric mucous membrane to become active, but also to sustain it in activity. If egg-albumin be administered so as to evoke the psychical influence, a well-marked and sustained secretion resulted. Chischin accordingly explains the usual process of secretion as occurring in the following manner:—At the time of taking food the first flow of gastric juice is determined by the reflex psychical influences involved in taking food. The digested proteids are able later to evoke a secretion, at a time presumably when the psychical influence begins to wane.

According to these experiments, then, we may assume that small quantities of peptone may be normally formed in the stomach, and,

[1] "Ueber die Function des Magens," *Verhandl. d. Cong. f. innere Med.*, Wiesbaden, 1893.

[2] Inaug. Diss., St. Petersburg, 1894. Reported in *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xxv.

becoming absorbed there, in some way influence the epithelium so that secretion results. The exact course of this absorption is a matter of some difficulty as far as the secretory epithelium is concerned, but it is yet more difficult to comprehend how peptone absorbed and changed in the intestinal wall should influence secretion in the stomach. If, as Schiff[1] long ago suggested, the absorption of these products should assist in building up the precursor of pepsin, we could more easily see the importance of these products passing to the secreting cells. Schiff, however, emphasised dextrin as being pre-eminently a "peptogenous" substance. Chischin finds that it does not evoke secretion.

The conditions of formation of the ferments of the gastric juice.—(*a*) *The conditions of the formation of pepsin.*—As previously mentioned, Brücke[2] had noticed that the pepsin present in the gastric mucous membrane was not yielded entirely to one extraction, and Ebstein and Grützner[3] pointed out that the peptic activity depended considerably upon the manner in which an extract was prepared. An extract made by treating the gastric mucous membrane with hydrochloric acid was much more powerful than one obtained by subjecting the mucous membrane to the action of glycerin. That which was not extracted by glycerin, Ebstein and Grützner regarded as a compound of pepsin with the proteid matter of the cells, this compound yielding pepsin on subjection to the influence of acid, or to the action of sodium chloride. Schiff[4] had also remarked, that if a dilute acid be added to the stomach and left for some weeks, the extract becomes gradually richer in peptic activity. Schiff accounted for this by assuming the existence of a precursor of pepsin in the cells of the mucous membrane, which gradually became converted into pepsin by the acid. This he called *propepsin.* In both cases the observers were dealing undoubtedly with some substance which yielded pepsin, and to this substance the name *pepsinogen* has since been applied. Ebstein's and Grützner's test for the existence of this substance was the fact that it was not dissolved by glycerin as was pepsin, and yet would yield pepsin on treatment with acid. But it was soon found that there were difficulties in differentiating pepsin from its precursor in this manner. Von Wittich[5] pointed out that when fibrin is placed in a glycerin extract of pepsin, the fibrin absorbs the pepsin, and will only yield it again to fresh treatment with acid. Ebstein and Grützner[6] further showed that even coagulated egg-albumin would do this. Thence it followed that the proteids of the gastric mucous membrane might fix the pepsin, and that a glycerin extract of the mucous membrane might be an extract of such pepsin as was not fixed by the proteids. It was necessary, therefore, to find some more definite test of the presence of pepsinogen. This was supplied by Langley,[7] who found that sodium carbonate had a powerfully destructive effect on pepsin, but a much less marked action on certain extracts of the mucous membrane from which pepsin could be derived. These extracts, therefore, were held to contain the zymogen. He also inferred that the gastric glands contained the ferment in the zymogen state, as they did not contain any appreciable amount of

[1] "Leçons sur la physiologie de la digestion," 1867, tome ii.
[2] "Vorlesungen," 1874.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. viii.
[4] *Ibid.*, 1877.
[5] *Ibid.*, 1872, Bd. v.; 1873, Bd. vii.
[6] *Op. cit.*
[7] "The Histology of the Mammalian Gastric Glands, and the Relation of the Pepsin to the Granules of the Chief Cells," *Journ. Physiol.*, Cambridge and London, 1882, vol. iii.

pepsin, but would yield the same under appropriate treatment. The differentiation of the one from the other was further advanced by Langley and Edkins.[1] They confirmed the observation, that alkalies and alkaline salts rapidly destroy pepsin. The conditions influencing the rate of destruction by sodium carbonate were found to be the strength of the solution of the alkaline salt, the time during which it is allowed to act, the temperature of the mixture, and finally the amount of proteids present. By mere neutralisation of an acid solution of pepsin, a considerable amount might be destroyed. If equal volumes of an extract of pepsin and of a 1 per cent. solution of sodium carbonate were mixed, in fifteen seconds as much as 97 per cent. of the pepsin might be destroyed. The greater the amount of proteid present, the greater the amount of sodium carbonate necessary to cause destruction. The difference between pepsin and pepsinogen in their behaviour with different reagents is merely one of degree. Pepsinogen is destroyed also by alkalies, but the destruction is so slow as compared with that of pepsin, that this reaction furnishes a useful method of distinguishing the one from the other. Since the aqueous extract of the gastric mucous membrane of a fasting animal loses but very little peptic power on brief treatment with 1 per cent. sodium carbonate, it follows that pepsinogen, but little or no pepsin, is present in the gastric glands in hunger. Schiff stated that "propepsin" was slowly converted into true pepsin. Langley and Edkins found that the conversion of pepsinogen into pepsin is one of great rapidity. All the pepsinogen present in an aqueous extract of a cat's gastric mucous membrane may be converted into pepsin by treatment with 1 per cent. hydrochloric acid in sixty seconds. With reference to the point as to whether pepsin is present in the gland cells during digestion, no definite result was arrived at. Pepsin can be obtained from the gastric mucous membrane of an animal in digestion, but not invariably, and such as is found may have been produced by the acid in the lumen of the tubes affecting the pepsinogen in the contiguous chief cells. In the œsophagus of the frog, where no acid is secreted, but only ferment, injection of commercial peptone causes no accumulation of pepsin in the gland cells. Carbonic acid destroys pepsinogen more rapidly than pepsin; but if only a small quantity of peptone is present, there is practically no destruction. Finally, it is observed that both pepsin and pepsinogen are rapidly destroyed on heating to a temperature of 55°–57° C.

(*b*) *The conditions of formation of rennin* (*rennet-ferment*).—An enzyme which has the property of causing milk or the separated caseinogen to undergo coagulation, is found in the stomachs of almost all animals. As regards the secretion of rennin, there is an important resemblance to that of pepsin, inasmuch as, in the case of the former, there is a precursor of the actual ferment existent in the glands of the stomach which has the power, under the influence of acid, of producing the active enzyme. It was in the case of the rennin that it first was shown that many of the ferments of the alimentary canal have a zymogen stage. Hammarsten,[2] in 1872, pointed out that the gastric glands of many animals contain rennet-zymogen, but do not contain rennet-ferment. The zymogens of pepsin and trypsin were not

[1] "Pepsin and Pepsinogen," *Journ. Physiol.*, Cambridge and London, 1886, vol. vii.
[2] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872.

described till some years later. Langley[1] showed that the method of separating pepsin from pepsinogen was applicable also to the rennin, since rennet-ferment was destroyed by sodium carbonate, whilst rennet-zymogen is affected much less powerfully. Hammarsten has also shown that the amount of rennet-ferment that can be extracted from the cardiac end of the stomach is proportionally much greater than that obtainable from the pyloric mucous membrane. Grützner[2] has shown that in the gastric glands of the dog, the rennet-ferment diminishes in amount during digestion, and that the amount of diminution runs parallel to that of pepsin. It seems that where pepsin is greatest in quantity, there also is rennet-ferment most abundant, and it seems probable that the granules of the chief cells contain the zymogens both of rennet-ferment and pepsin. We cannot say whether the granules are of one kind or whether there are separate forms of granules for the separate ferments. But though in general the zymogen of the rennet-ferment, and not the actual ferment, is existent in the gastric cells, yet in some cases, *e.g.* the calf and sheep, the zymogen is presumably in a

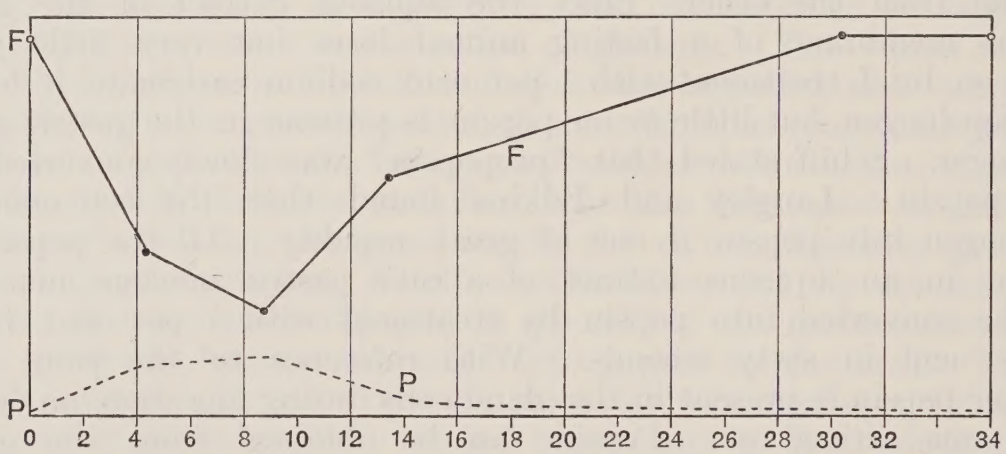


FIG. 44.—The figures at the abscissæ on the base line refer to the number of hours elapsed since the last meal. The length of the ordinates indicates the amount of pepsin yielded at any time. F is the record of variation in the fundus mucous membrane, P of variation in the pyloric mucous membrane.—After Grützner.

much less stable condition, for a watery extract of the stomach of these animals yields rennet-ferment in large quantities. As regards the differentiation of the rennet-ferment from the proteolytic, they can be separated from one another by chemical means, although we have no morphological signs of their distinction. Hammarsten's method of separating the two ferments chemically depends upon the fact that the gradual addition of lead acetate precipitates the pepsin sooner than the rennin.[3]

The variations in the amount and composition of gastric juice during the course of digestion.—The amount of pepsin that can be extracted from the mucous membrane has been estimated by Grützner.[4] He compared concurrently that obtained from the fundus with that yielded by the pyloric region. In the above chart (Fig. 44), which shows the chief variation during the lapse of some hours after a meal, the most

[1] "On the Destruction of the Ferments of the Alimentary Canal," *Journ. Physiol.*, Cambridge and London, 1882, vol. iii.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvi.

[3] The conditions of formation of the hydrochloric acid of the gastric juice are treated of in a preceding article (see pp. 351 *et seq.*).

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xx.

striking feature is the absence of coincidence between the pepsin contents of the pyloric and the fundus region of the stomach.

In general, it may be pointed out that the maximal yield of pepsin from the pyloric region is at the same interval after ingestion of food as marks the minimal yield of pepsin from the fundus.

A great number of observations have been directed to estimating the acidity of the contents of the stomach at different intervals after a meal has been taken. Gastric juice commences to be secreted almost as soon as suitable food enters the stomach. For a time the acid juice merely neutralises the alkalinity of the food and saliva, and the hydrochloric acid combines with various food substances, so that free hydrochloric acid does not occur till after an appreciable interval. Von den Velden[1] states that free hydrochloric acid cannot be detected until three-quarters of an hour after a meal is taken. Richet[2] states that in the human stomach the acidity gradually increases during digestion, and that it is apparently independent of the quantity of fluid taken. Towards the end of digestion he finds that the total acidity of the stomach contents may be further increased, but this is to be referred to the production of organic acids by the decomposition of the food. He also points out that the feebler the activity of the juice, the greater the amount of organic acid liberated. Chischin's[3] observations give precise details of the course of digestion with different foods. The annexed diagram (Fig. 45) shows the course of the production of hydrochloric acid in an isolated portion of the fundus, when the animal was fed with mixed food, comprising milk, meat, and bread. The animal did not undergo the "psychical" stimulation of the food, or the maximal percentage of hydrochloric acid would probably have been in the first hour, instead of in the second or third. If meat alone be given to such an animal under similar conditions, the maximal acidity occurs in the first hour. With mixed food the digestive power averages 3·5 mm. (Mette's method of estimation by columns of coagulated egg-white);[4] with simply meat food, about 4 mm. With bread alone as food, the duration of secretion was found to be more protracted, but the digestive strength was much greater, varying between 5·22 mm. and 7·56 mm. The digestive power was very marked in the first hour, increased further in the second hour, and remained high both in the third and fourth hours. With milk, the course of secretion is much more irregular. The digestive power is moderately high at first, but sinks, after the first hour, about one-half. It remains at this strength for the third and fourth hours, but in the fifth hour increases again to the original strength, and may, in the sixth hour, even go

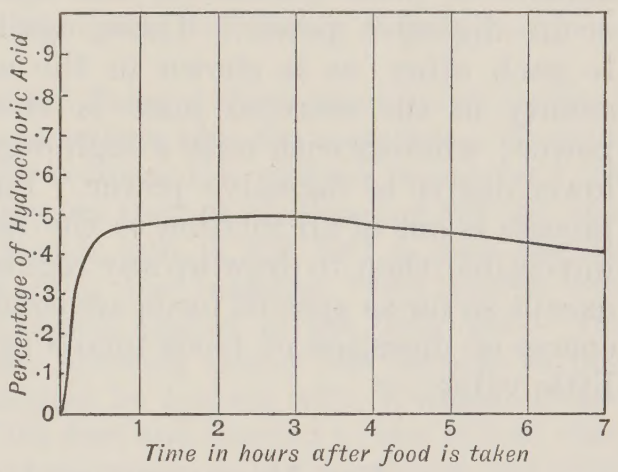


FIG. 45.—Chart showing acidity of gastric juice after feeding with mixed food (300 c.c. milk, 50 grms. meat, and 50 grms. white bread). The animal was not subjected to the "psychical stimulation" of the food.

[1] "Zur Lehre von der Wirkung des Mundspeichels im Magen," *Ztschr. f. physiol. Chem.*, Strassburg, 1879, vol. iii.

[2] *Op. cit.* [3] *Op. cit.* [4] For Mette's method, see p. 325.

beyond this. In another series of experiments the animals were fed with peptone (Chapoteaux). This, according to Chischin, was equivalent to reviewing the later stages of digestion, from the time when peptone began to be formed in any quantity in the stomach. The noticeable point about the results in these last cases is, that there is presented a great contrast to feeding with such a primary proteid as egg-albumin. With peptone, the juice becomes secreted in large quantities at once, its acidity is high, and its digestive power well marked.

It is obvious, therefore, that the nature of the food has an important influence on the course and nature of the secretion. This has been drawn attention to by Khigine,[1] who classes the different foods mentioned above in different orders. He has also pointed out that the amount of juice secreted is not necessarily proportional to its acidity or its digestive power. These, again, are not necessarily proportional to each other, as is shown in the case of bread as food, when a low acidity in the secreted juice is shown, but a high degree of peptic power; whereas with milk a high degree of acidity is shown, but a much lower degree of digestive power. Finally, the duration of the digestive process is out of all relation to the strength of the secreted juice. It is impossible, then, to draw up any regular scheme of the course of digestion, except so far as specific foods are concerned, observations based upon the course of digestion of foods mixed in arbitrary proportions being of but little value.

The Mechanism of Pancreatic Secretion.

The histological appearances of the different secretory conditions of the pancreas.—The pancreas consists of secretory alveoli, between which are here and there seen masses of cells of a different character, and having no connection with the proper secretory channels of the gland. These masses of cells are presumably not connected with the ordinary processes of the secretion of a digestive juice, and the following account will therefore be confined to the typical secretory alveoli.

If a small portion of the pancreas of an animal be examined in the living state, it will be found to consist of many secretory alveoli, and these secretory alveoli of cells contain numbers of discrete granules. It is generally found that whatever digestive stage the animal is in, there exists an outer zone in the alveolus free from granules. This is not, however, invariably the case. Ordinary stains, such as hæmatoxylin and carmine, are found to colour this outer zone more deeply than the rest. This is in conformity with the usual rule, that such stains do not deeply colour the secretory granules of cells, or the substance formed by their breaking down. If the cells are macerated for a few days in neutral ammonium chromate, a radial fibrillation is revealed[2] in this outer zone. The addition of water to the fresh gland causes the granules to disappear, and dilute alkalies produce this result even more rapidly. Acids, either mineral or organic, cause the distinction between the two zones to be lost, the whole cell becoming clear. By hardening the gland in solutions of osmic acid, or in the vapour of osmic acid, the granules may be preserved.

[1] "Études sur l'excitabilité sécrétoire specifique de la muqueuse du canal digestif" *Arch. de sc. biol.*, St. Pétersbourg, 1895, vol. iii. p. 5.

[2] Heidenhain, Hermann's "Handbuch," Bd. v. Abth. 4.

Although, in the resting condition of the gland, an outer border free from granules is evident, this is still more manifest in the exhausted condition. The granules may then be so reduced in number as to form small aggregations at the luminal borders only of the cells.

As is the case in the stomach, there is reason to believe that the granules are concerned with the specific secretion of the gland, the amount of granules determining the activity of an extract.

The above described changes in the cells were first observed in the living pancreas of the rabbit by Kühne and Sheridan Lea.[1]

Methods of obtaining pancreatic juice.—The methods that have been adopted to procure a supply of pancreatic juice involve one of the following procedures—(*a*) Fixing a cannula into the duct of Wirsung; (*b*) opening the duct and connecting it with the body wall; (*c*) cutting out a piece of the intestine in which the pancreatic duct opens, and fixing this to the body wall.

C. Bernard [2] adopted the first method, fixing a silver cannula into the duct. Heidenhain [3] introduced antiseptic precautions into the operation. He made an incision in the linea alba midway between the xiphoid process and the umbilicus. The duodenum was drawn out through the opening and the duct carefully sought for. This being found, into it was tied a glass cannula of about 6–18 cms. in length. Around the intestine were placed two temporary ligatures, keeping the gut closely applied to the body wall. The opening was found to gradually close, allowing simply the cannula to pass through. The second method was adopted by Ludwig with Weinmann,[4] and Bernstein.[5] They found and opened the duct and inserted a piece of lead wire, on the one hand, towards the papilla pancreatica in the duodenum, the other end passing up to the gland substance. This wire did not fill the lumen, and thus the flow was still permitted. The third method, which is due to Heidenhain and adopted for permanent fistulæ, consists in resecting the small portion of the intestine which contains the papilla pancreatica, and joining the ends of the main gut above and below. The piece of intestine is slit up, the mesenteric surface is attached to the body wall, and thus the juice can be obtained. Pawlow varied this method by not resecting the whole tube of the intestine. He cut out a quadrangular piece, including the pancreatic papilla, and ligatured this into the body wall.

By these different methods natural pancreatic juice may be obtained. After a time the juice becomes somewhat altered; it retains, however, its ferment activity in a marked manner throughout.

The influence of the nervous system upon pancreatic secretion.—Our knowledge has lately been considerably extended in respect of the precise influence of nervous impulses upon pancreatic secretion. The following statements summarise our chief knowledge up to the most recent researches upon the subject.[6]

1. After division of the nerves, proceeding to the gland, secretion is set up and apparently increases. This was affirmed by Bernstein.[7]

2. Secretion can be set up by stimulation of the medulla oblongata, or, if already in progress, can be increased.[8]

3. The medulla oblongata must not be regarded as exclusively the

[1] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, N.F., Bd. i.

[2] "Mémoire sur le pancréas et sur le rôle du suc pancréatique," *Compt. rend. Acad. d. sc.* Paris, 1856.

[3] Hermann's "Handbuch," Bd. v.

[4] *Ztschr. f. rat. Med.*, Bd. iii.

[5] *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, Leipzig, 1869.

[6] Cf. Heidenhain, Hermann's "Handbuch," Bd. v. Abth. 4.

[7] *Op. cit.*

[8] Heidenhain, *Arch. f. d. ges. Physiol.*, Bonn, 1875.

centre for pancreatic secretion, as, after its separation from the cervical spinal cord, the secretory process can continue, although with diminished intensity.

4. The nerves proceeding to the pancreas do not seem to have the same direct influence upon the secretion that the nerves to the salivary glands possess.

5. Stimulation of the central end of the divided vagus, according to Bernstein,[1] or of sensory nerves in general (*e.g.* cutaneous), according to Afanassiew and Pawlow,[2] may inhibit the secretion, provided the pancreatic nerves are intact. This, Heidenhain regards as due to vascular changes.

Pawlow[3] found that the administration of atropine stopped the secretion frequently, but not in all animals (*e.g.* in dogs but not in rabbits), and Heidenhain observed that the administration of pilocarpine caused a sluggish secretion of a concentrated juice.

The later experiments of Pawlow and the St. Petersburg school have greatly amplified our knowledge of the nervous influence. In Pawlow's further researches he observed the effects of nerve stimulation upon dogs prepared for experiment in two different ways. In the first case the dog had a permanent pancreatic fistula prepared, one vagus in the neck was also divided. The stimulation of the peripheral stump of the vagus was performed some five days after the section, at a time when certain fibres in the vagus had degenerated. In the second case the vagus was cut through in the neck, and after three or four days the animal was prepared for experiment by the performance of tracheotomy, section of the spinal cord just below the medulla oblongata, and the preparation of a fresh pancreatic fistula. In both these cases stimulation of the peripheral end of the vagus causes secretion from the pancreas. Moreover, stimulation of the intact vagus also produces this result, and even if neither vagus is divided a more or less pronounced secretion ensues. Certain differences are observable, however, between the general effects in the two cases. In the first case more secretion was produced, this being comparatively watery in character and greatest in amount at the commencement of stimulation. These differences are probably accounted for by the general low blood pressure in the second case. The pressure of the secretion was found by Pawlow to be lower than the corresponding blood pressure, and it was noted that vagus stimulation in one case still caused a secretion, although the blood pressure was reduced by bleeding practically to nil. Frequently the secretion would end with the lowering of the blood pressure, but nevertheless the one experiment is sufficient to establish the independence of the secretion of the blood pressure. The action of atropine is to cause a marked influence on the effects of nerve stimulation, though complete cessation of the secretion is not produced. Reflex effects can be produced on the secretion, which do not correspond to the effects upon the blood vessels. Stimulation of the central stump of the lingual or of the vagus nerve will produce such reflex effects. If, at the commencement of the experiment, either no secretion or a slight secretion occurs, with the first stimulation of sensory nerves either a commencement or an increase of the secretion results. After the stimulation ceases this lessens. If after the first stimulation the

[1] *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, Leipzig, 1869.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvi.
[3] *Arch. f. Physiol.*, Leipzig, 1893, Supp. Bd.

secretion is still fairly marked, a further stimulation will result in inhibition of the secretion, which inhibition ends with the stimulation provoking it. The spontaneous secretion that is sometimes observed before the experiment begins, is stopped by cutting both vagi, and is therefore due to impulses proceeding from the upper portion of the cervical spinal cord or the medulla oblongata. Pawlow also points out the importance of the circulation in general for the secretion. A brief stoppage of the blood stream caused a cessation of the flow, and an anæmic condition of the gland resulting from reflex nervous influence caused a similar cessation. The latent period relapsing before the secretion resulting from stimulation becomes obvious, is, according to Pawlow, two to three minutes, but later observers such as Mette [1] and Kudrewetzky [2] regard it as somewhat longer, namely, from four to six minutes. Mette in addition found that, though previous observers (Lewaschew, Heidenhain) had stated that the proteolytic ferment failed in the pancreatic juice of dogs which had fasted five or six days, yet it was continuously obtainable by stimulation of the vagus. Gottlieb [3] confirms the old observation, that stimulation of the divided vagus at the central end causes inhibition of the secretion, and he refers this result to general spasm of the abdominal blood vessels. Another contribution to the study of the inhibitory influences on the pancreatic secretion has recently been made by Popielski.[4] It had been previously noticed by Mette and Kudrewetzky that the secretion caused by stimulating one vagus could frequently be stopped by stimulating the other vagus. Hence it was inferred that antagonistic fibres passed in these nerves. Stimulation of such fibres may bring about sometimes a lengthened latent period, sometimes total inhibition of the flow. Mette regarded this as due to the existence of vaso-constrictor fibres, Kudrewetzky to the presence of specific fibres inhibiting the secretion. Popielski endeavoured to elucidate this point. He found that a secretion evoked by peripheral stimulation of the vagus could later, by a repetition of the stimulation of the same nerve, be interrupted. The interruption started some seven seconds after the stimulation commenced, and lasted about the same interval beyond the cessation of stimulation. This inhibition also follows from stimulation of the other vagus, as previously observed, and is best shown when the exciting current is not too strong. The branch of the vagus which lies behind the œsophagus in the thoracic cavity is that concerned with changes in the secretory activity of the pancreas. Dolinski [5] had previously observed that the introduction of acids into the duodenum produces a flow of pancreatic juice (see next section). Popielski made use of this fact to see how far the secretion thus excited could be inhibited by nerve stimulation. He found that a secretion so produced was inhibited with perfect regularity by stimulation of the vagus. Stimulation of the vagus, after secretion evoked by pilocarpine, produced the same result. Popielski points out that there are three ways in which inhibition of the flow of pancreatic juice can be brought about—

1. By stimulation of vaso-constrictor fibres.

[1] *Arch. f. Physiol.*, Leipzig, 1894, Supp. Bd. [2] *Ibid.*, 1894.
[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1895, Bd. xxxiii.
[4] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1896, Bd. x.
[5] *Arch. de sc. biol.*, St. Pétersbourg, 1895, vol. iii.

2. By constriction of the lumen of the duct, resulting from contraction of its smooth muscle.

3. By action of special nerve-fibres inhibiting secretion.

The first hypothesis is improbable, since stimulation of the splanchnics does not cause the same cessation; and, moreover, there is reason to doubt the existence of vaso-constrictor fibres in the vagus.[1]

The second hypothesis will not hold, when it is considered that physostigmine produces duct constriction, but at the same time increases the secretion.

Before examining in detail the third supposition, Popielski endeavoured to see how far special secretory fibres can be anatomically isolated. If the larger branches of the vagi lying on the stomach, or those branches which pass towards the liver, be divided, stimulation of the vagus has the same influence upon pancreatic secretion. The impulses pass therefore along some of the finer nerve branches running in the subserous coat towards the pyloric region of the stomach. If the duodenum be cut through near the pylorus, stimulation of the vagus has no effect. If the duodenum be cut across lower down, the vagus effect is apparent. Stimulation of the lower cut edge of the duodenum in the first case provokes secretion, and if the main mass of nerves passing with the vein into the gland be stimulated (especially those lying at the upper side of the vein), a secretion is evoked without marked latent period, and uniform in character. This secretion is inhibited by the simultaneous stimulation of the vagus in the thoracic cavity. The inhibition comes about, then, either by impulses passing along nerve-fibres to the gland-cells, or affecting some nerve-centre. Popielski finds that if the vagi and sympathetic nerves be cut, a reflex secretion is still evoked by placing hydrochloric acid in the duodenum. The reflex centre, he thinks, then, must be in the abdominal cavity. He considers it probable that such a centre exists in the region of the pylorus, since, if the duodenum be cut through near the pylorus, the introduction of hydrochloric acid is then without effect. If the pylorus be separated with the duodenum, hydrochloric acid will then, however, have the usual effect of causing pancreatic secretion. Popielski considers, however, that such a reflex centre is not furnished by the semilunar ganglion.

If these observations are correct, we can assume the existence of secretory and inhibitory nerve-fibres, both running in the vagi, and it seems probable, from the differences of latent period which result from stimulation in different regions, that the inhibitory impulses passing along the vagus do not act directly on the cells of the gland, but on some centre which has a controlling influence on the process of secretion. Popielski's reasons for regarding the semilunar ganglion as probably not furnishing such a centre, seem insufficient. The fact that Bernard found an increased secretion after extirpating this, can be explained, on the analogy of the salivary gland, as a paralytic secretion. There is some evidence that the inferior mesenteric ganglion may also act as a centre for reflex action, and if so, it seems less improbable that a similar reflex centre for the pancreatic secretory processes may be referred to the semilunar ganglion. Should such a centre exist, it is undoubtedly subject to influences proceeding from the higher centres by means of the vagi.

Though there is difficulty in admitting the existence of a controlling

[1] François-Franck.

centre for the pancreatic secretion in the semilunar ganglion, there is even greater difficulty in associating such a centre with any other neighbouring structure, or in admitting that, as Popielski considers, it may be placed in the walls of the pylorus.

The conditions under which local stimulation provokes the flow of pancreatic juice.—As stated in the last section, a secretion of the pancreatic juice, dependent upon integrity of the nerve connections, can be brought about by the action of certain substances upon the mucous membrane of the stomach or duodenum. Thus it was long ago noticed that injection of ether into the stomach will cause a flow of pancreatic juice, the juice having characters corresponding to the particular stage of digestion in which the flow is brought about.[1] More recently, other substances have been found to similarly affect the secretion. If mineral acid, or even organic acids such as acetic and lactic, be brought into contact with the duodenal mucous membrane, a secretion will result. Since alkaline substances have not the same effect, Dolinski[2] considers that the acid products of gastric digestion bring about their own neutralisation by inducing a flow of alkaline pancreatic juice when they enter the small intestine. Dolinski also found that fat excited reflexly a pancreatic secretion, and that alcohol was also effective in this direction, but only to a moderate degree. Gottlieb[3] agrees that reflexly induced secretion starts generally by stimulation of the duodenal mucous membrane. Becker[4] studied the effect upon the secretion of the introduction into the stomach of distilled water and of various salts. The salts employed were various alkaline salts, Carlsbad salts, sodium chloride, and "Essentouck" mineral water. Becker found that distilled water exalted the secretion, whilst salts, especially alkaline salts, diminished it, both in amount and in proteolytic power. Sodium chloride in smaller doses was indifferent, in larger doses it behaved as the alkaline salts. The better the absorption the more marked the secretion. Water containing carbonic acid is more easily absorbed than simple distilled water, and, correspondingly, the former excites a more plentiful secretion than the latter.

We see, then, that the ordinary progress of the food can account for the secretion normally appearing; further, that the acid contents of the stomach, when passed into the duodenum, may cause a powerful secretion, and that alkaline salts in the stomach diminish the secretion.

The ferments of the pancreatic juice and their antecedents.—Extracts made from the pancreas of many animals, and the pancreatic juice obtained by the establishment of fistulæ, possess the power of changing different foodstuffs. Heidenhain[5] showed in 1875 that there could be obtained from the pancreas a substance from which the proteolytic ferment could be derived, but which did not actually possess proteolytic activity. This substance he called "zymogen," but since we are acquainted with substances having similar relations to other enzymes, it is better to retain the name zymogen for the whole class, and to refer each individual precursor by a name associated with the particular ferment. We thus speak of the particular zymogen of the proteolytic enzyme of the pancreas as trypsinogen.

[1] Kühne, "Lehrbuch der physiol. Chem." [2] *Op. cit.* [3] *Op. cit.*
[4] *Arch. de sc. biol.*, St. Pétersbourg, 1893, vol. ii.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. x.

Heidenhain established definite characters distinguishing trypsinogen from the actual enzyme, and showed that in some respects their behaviour was similar. The chief relations of the zymogen to the ferment are as follows:—

1. Trypsinogen is soluble in glycerin. Some glycerin extracts of pancreas have no ferment activity, since the ferment is in the condition of zymogen, but if such glycerin extracts, dissolved in 1 per cent. sodium hydrate, be diluted with distilled but not boiled water (this being largely devoid of dissolved air), especially if digested for a time at 40° C., it will become active.

2. If an inactive glycerin extract of fresh pancreas be dissolved in sodium carbonate, 1 to 2 per cent., the passing through it of oxygen will cause the same to become active.

3. Platinum black will, according to Podolinski,[1] also render the inert extract proteolytic.

4. The converse of the change brought about by the influence of oxygen may also occur, for, through the deprivation of oxygen, activity becomes lost.

5. If fresh pancreas be mixed with the same weight of 1 per cent. acetic acid for ten minutes, and then placed in glycerin, a very active extract will be obtained. The acetic acid converts the trypsinogen into trypsin. According to Kühne,[2] trypsin is also formed from the zymogen by warming with alcohol.

The amount of trypsin that can be obtained from an extract varies with the histological condition of the gland. When the luminal zone is of considerable width, a greater amount of proteolytic activity is shown than when it is much reduced. We are justified in associating the ferment with the granules seen in the cells.

Sodium carbonate may be regarded as an adjuvant to the action of trypsin. Kühne[3] showed that it worked best in solutions of the strength of 1 per cent. Edkins[4] proved that sodium chloride has a beneficial influence on the digestion of fibrin by pancreatic extracts, and it may be noted that a large amount of the sodium carbonate associated with the pancreatic secretion must be converted into sodium chloride in the duodenum. Ewald[5] states that digestion of fibrin at the instance of trypsin can proceed in the presence of 0·3 per cent. of hydrochloric acid, but, on the other hand, the prolonged action of dilute acids has been shown by Langley[6] to be destructive of trypsin. If a glycerin extract of pancreas be warmed for two and a half hours in 0·05 per cent. hydrochloric acid, its proteolytic powers are appreciably curtailed. The diastatic ferment has not had the same study bestowed upon it as the proteolytic. It contrasts with this latter in that there is no further enhancement of its activity by treatment with such reagents as convert trypsinogen into trypsin. Liversedge[7] made observations in 1874, which suggested the existence of a diastatic zymogen, but the possibility of micro-organic change influencing his experiments was, as pointed out by Gamgee,[8] not eliminated. According to his observations,

[1] Breslau, 1876.
[2] *Verhandl. d. naturh.-med. Ver. zu Heidelberg*, 1876, N. F., Bd. i.
[3] *Ibid.*, Bd. i. [4] *Journ. Physiol.*, Cambridge and London, 1891 Bd. xii.
[5] *Ztschr. f. klin. Med.*, Berlin, Bd. i.
[6] *Journ. Physiol.*, Cambridge and London, vol. iii.
[7] *Journ. of Anat. and Physiol.*, London, 1874, vol. viii.
[8] "Physiological Chemistry," vol. ii. p. 207.

the zymogen is not soluble in water, thus contrasting markedly with other zymogens. We must regard the existence of a precursor in this case as doubtful, though it is undeniably possible that in the living cell an antecedent state of the ferment exists, adapted to storage of the ferment; in that case the mere destruction of the cell might involve the breaking down of this hypothetical zymogen, on account of the precursor of the diastatic ferment being less stable than that of the proteolytic.

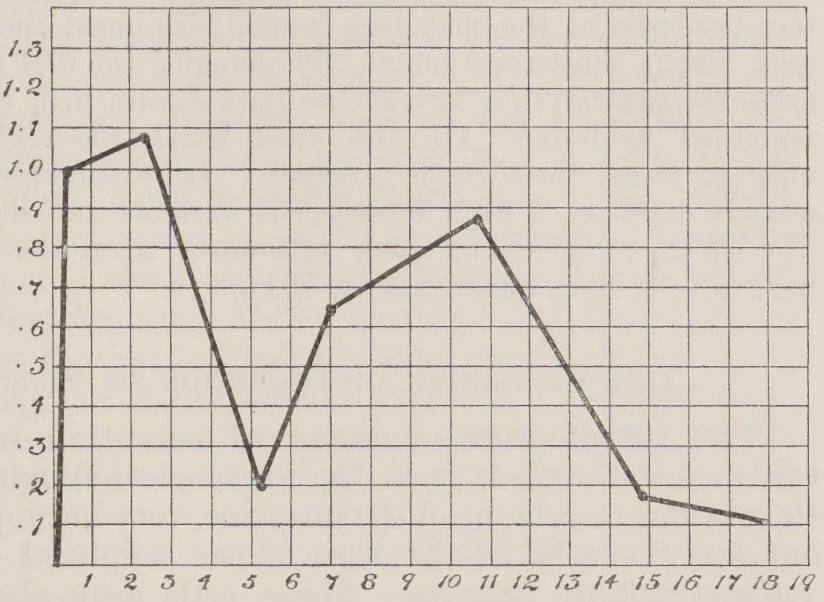


FIG. 46.—Chart of the course of secretion of pancreatic juice. The abscissæ correspond to hours; the ordinates correspond to c.c. of juice.—After Heidenhain.

There is also no evidence of any zymogen of the fat-decomposing ferment, pialyn.

Finally, it has been found that extracts of pancreas and the pancreatic juice itself[1] have the power of inducing a clot in milk, probably by the agency of some specific enzyme in the juice.

The variations in the composition and amount of pancreatic juice during digestion.—From the earlier experiments of Bernstein,[2] and those of Heidenhain,[3] it appears that the flow of pancreatic juice has somewhat the following course:—

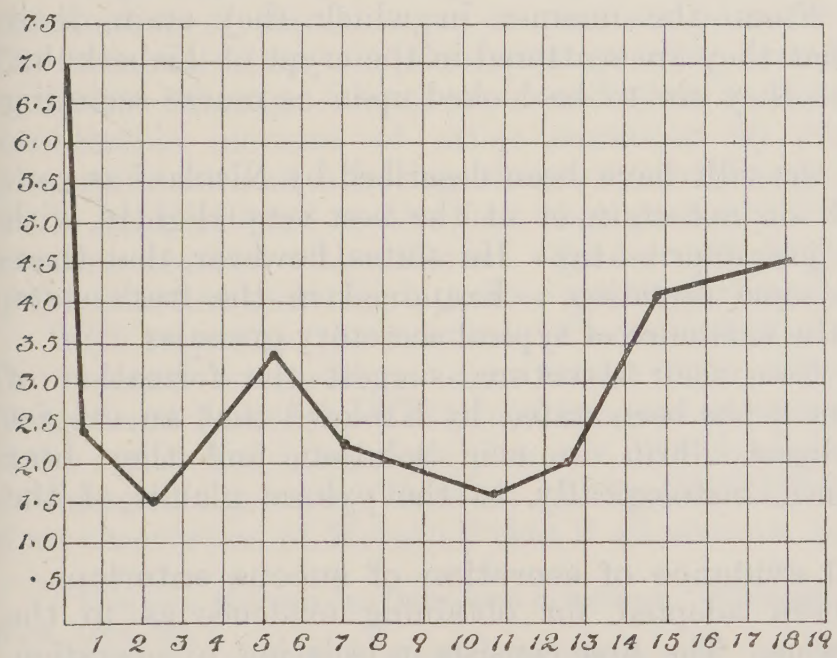


FIG. 47.—Chart of the percentage composition of the flow of pancreatic juice. The abscissæ correspond to hours; the ordinates to percentage of solids.—After Heidenhain.

Before a meal is over there commences a secretion, which reaches a maximum not later than the third hour. Then the secretion sinks to about the sixth or seventh hour, and yet again increases to the ninth or eleventh; thence it sinks gradually to about the eighteenth and twentieth. The quality of the juice varies inversely as the quantity. When one rises the other falls. The accompanying diagrams (Figs. 46 and 47) illustrate these variations.

[1] Halliburton and Brodie, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx.
[2] *Op. cit.*
[3] Hermann's "Handbuch," Bd. v.

It has been pointed out by Mette that during normal digestion there is a certain independence between the secretion of ferment and the secretion of water. Observations have also been made by Wassilieff[1] on the influence of food in causing changes in the activity of the juice. He found that the maximum of secretion was in the first two hours, with meat diet in the first hour, and milk diet in the second hour. By changing the diet from meat to bread and milk, the proteolytic action of the juice diminished, whilst the diastatic action remained unaltered. On the other hand, when changing from bread and milk to meat, these were reversed. It is therefore to be noted that the relative quantity of both ferments is variable and dependent upon the food. The effects produced by other substances upon the flow of pancreatic juice have already been mentioned (p. 551).

The Mechanism of Secretion of Succus Entericus.

The histological evidence of secretion in the intestine.—The evidence of secretion from the histological standpoint is, in the case of the mucous membrane of the intestine, very incomplete. Paneth[2] pointed out that the cells at the base of the crypts of Lieberkühn frequently contain definite granules. These cells were also studied by Nicolas,[3] who noticed different phases in the condition of the cells; thus, after secretory activity, he found them either free from or containing but few granules.

Hardy and Wesbrook[4] found that in fasting animals the granules were large and numerous, in well-fed animals comparatively few, and smaller than in the fasting state.

Bizzozero[5] regards the granules as mucigen granules. Schaffer[6] has also called attention to the fact that the cells containing them are goblet-shaped. From the manner in which they stain, their shape, and the fact that they are scattered in the crypt of Lieberkühn, it seems probable that they are to be looked upon as mucus-secreting cells.

The cells covering the villi have been described by Nicolas[7] as containing granules which do not stain, or at the best very slightly, with safranin (unlike those just referred to). He states, however, that these granules give rise to some secretion. Examined in the fresh state, the cells do not show the existence of typical secretory granules.

Brunner's glands, from their structure, suggest the formation of a mucous secretion,[8] but it has been stated by Krolow[9] that an extract of the glands will digest fibrin in acid solution, and they bear considerable resemblance, histologically, to the pyloric glands of the stomach.

The experimental evidence of secretion of succus entericus.—Two methods have been adopted for obtaining evidence as to the nature of succus entericus. The first consists in isolating, by operation, a piece of the intestine, and observing the nature of the liquid which

[1] *Arch. de sc. biol.*, St. Pétersbourg, 1893, vol. ii.
[2] *Arch. f. mikr. Anat.*, Bonn, 1888, Bd. xxxi.
[3] *Internat. Monatschr. f. Anat. u. Physiol.*, Leipzig, 1891, Bd. viii.
[4] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii.
[5] *Anat. Anz.*, Jena, 1888, Bd. iii.; *Atti d. r. Acad. d. sc. di Torino*, 1888–9.
[6] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1891, Abth. 3, Bd. c.
[7] *Op. cit.*
[8] Kuczynski, *Internat. Monatschr. f. Anat. u. Physiol.*, Leipzig, 1890, Bd. vii.
[9] *Berl. klin. Wchnschr.*, 1870, No. 1.

collects in the interior; the second, in making extracts of the intestinal mucous membrane and investigating the digestive properties of such an extract.

A method of permanently isolating a portion of the intestine was first devised by Thiry.[1] The abdomen of an animal having been opened, a piece of intestine was cut away from its continuity with the main gut without dividing the mesentery. The two ends of the main gut were then brought together and ligatured, so that union of the cut surfaces was brought about, the continuity of the intestine being thus re-established. The isolated portion of the gut was then closed by a ligature at its lower end, while the upper end was sewn into the incision in the abdominal wall, a blind sac being thus formed. Vella[2] modified this procedure by inserting the lower end of the isolated gut also into the abdominal wall; thus affording two openings for the separated intestine. This operation, performed with due antiseptic precautions, is of constant service at the present day, and is generally described as the establishment of a "Thiry-Vella" fistula.

Older observers, such as Bidder and Schmidt,[3] had ligatured off from the general tract short lengths of the intestine, and, after replacing them in the abdominal cavity for some hours, had examined the accumulated liquid.

The chief facts that have been brought to light by these methods are as follows:—In the absence of any stimulus, little or no secretion has been obtained, as a rule. Thiry,[4] with mechanical or electrical stimulation, obtained a thin yellowish alkaline secretion, albuminous in character. After food had been taken, although no previous secretion was manifest, some fluid began to form. According to Röhmann,[5] the introduction of starch, sugar, or peptone provokes intestinal secretion. The administration of pilocarpine results, according to Masloff,[6] in secretion. Gamgee,[7] however, found that it was possible to produce considerable increase of other secretions by the administration of pilocarpine without affecting the succus entericus to any extent. This result he attributed to the fact that probably different regions of the intestine reacted with different vigour to pilocarpine, the lower portion of the intestine secreting a greater quantity than the upper.[8]

With respect to the existence of nervous influences on the secretion, Thiry found no result to come about from stimulation of the vagi. Budge[9] and Lamansky[10] obtained increase of secretion after extirpation of the cœliac and mesenteric plexuses, but Adrian[11] did not succeed in obtaining this increase. Brunton and Pye-Smith[12] found, in confirmation of an observation of Moreau,[13] that if all nervous connections be severed

[1] "Eine neue Methode den Dünndarm zu isolieren," *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1864, Bd. i.

[2] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1881, Bd. xiii.

[3] "Die Verdauungssäfte und der Stoffwechsel," Leipzig, 1852.

[4] *Op. cit.*

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. ii.

[6] *Untersuch. a. d. physiol. Inst. d. Univ. Heidelberg*, 1882, Bd. ii.

[7] "Physiological Chemistry," London, 1893, vol. ii.

[8] That pilocarpine provokes an intense secretory charge in the crypts of Lieberkühn of the large intestine, is manifest from the experiments of Heidenhain (Hermann's "Handbuch," Bd. v.).

[9] *Verhandl. d. k. k. Leopold-Carol. Acad. d. Naturforscher.*, 1860, Bd. xix.

[10] *Ztschr. f. rat. Med.*, 1866.

[11] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1863, Bd. iii.

[12] *Rep. Brit. Ass. Adv. Sc.*, London, 1874, 1875, 1876.

[13] *Compt. rend. Acad. d. sc.*, Paris, 1863, Bd. lxvi.

between higher centres and the mucous membrane by dividing the intestinal nerves, an accumulation of fluid takes place. Brunton and Pye-Smith also found that if the inferior ganglia of the solar plexus and their continuation along the superior mesenteric artery are left in connection with the gut, this accumulation does not take place.

L. Hermann[1] initiated a somewhat different method of investigating the secretion. A loop of intestine was separated from the main gut, and its ends joined so as to form a confluent ring. This was replaced in the intestine, and its contents examined after some weeks. These contents were found to consist of solid material, and it was presumed that this represented the inspissated juice. Blitstein and Ehrenthal[2] continued these experiments, and came to the conclusion that the solid mass found had its origin in two sources; the first being the intestinal fluid, and the second detached intestinal epithelial cells. They noticed micro-organisms also to be present. Fr. Voit,[3] who simply sewed up the ends of an isolated loop, found, after the lapse of three weeks, a yellowish-grey mass, in which he recognised no epithelium, and which he regarded as simply inspissated juice. The nature of the fluid excreted in the Thiry-Vella loop has been frequently examined. It is of a yellowish colour, and contains albumin, and also a rather large amount of sodium carbonate. It possesses certain ferment-powers, though with regard to these there is considerable divergence of statement. Thiry[4] found it to dissolve fibrin, but not to affect other proteids. Masloff[5] found it to act feebly on starch, but not on proteids.

Funke[6] stated that starch injected into isolated loops is not converted into sugar. Later observers,[7] experimenting by the above methods, agree that starch is converted into sugar, and Röhman's experiments suggest a greater diastatic activity in the upper part of the intestine than the lower. This observer also finds, as Paschutin[8] had previously pointed out from experiments with extracts, that the intestinal juice has the power of inverting cane-sugar. It is to be noted that this is, even markedly, the case, as Gamgee[9] points out, in animals which would have no opportunity, from the nature of their food, of utilising the enzyme causing such a change. Observations made recently by Pregl[10] on a Thiry-Vella fistula established in a lamb, have somewhat completed the knowledge that has accrued from this method of research. He found that the secretion was continuous, but it increased the first hour after food, and this went on to about the third hour. From a length of intestine of 72 cm. he obtained about 5 grms. of intestinal juice per hour; this rate of secretion diminished to the fifth hour, when it reached 3 grms. per hour, and remained at this rate for many hours after. He refers to the prolapse which occurs at first, and with which other observers have found difficulty, and points out that this is evidence of a catarrhal condition, which itself would account for a certain amount of flow, although he failed to notice any difference between the juice reinforced by catarrhal

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xlvi.
[2] *Ibid.*, 1891, Bd. xlviii. [3] *Ztschr. f. Biol.*, München, 1893, Bd, xxix.
[4] *Op. cit.* [5] *Op. cit.* [6] "Lehrbuch."
[7] Gumilewski, *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxix.; Röhmann, *op. cit.*; Dobroslawin, "Beitr. z. Physiol. d. Darmsäftes," *Untersuch. a. d. Inst. f. Physiol. u. Histol. in Graz*, Leipzig, 1870; Lannois et Lépine, *Arch. de physiol. norm. et path.*, Paris, 1883.
[8] *Arch. f. Anat. u. Physiol.*, Leipzig, 1871. [9] *Op. cit.*
[10] *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxi.

exudation and the simple juice. Pilocarpine, he found, did not cause increased secretion, nor did electrical stimulation. He describes the juice as consisting of a yellow fluid, in which are suspended flocculi, staining deeply with eosin, and mainly mucous in nature. The alkalinity is marked. Albumin and globulin are present, and what he regarded as probably albumose. He also found a small amount of urea. The secretion had no action upon proteids. From starch paste was formed after twenty-four hours a fermentable sugar. This action was shown more powerfully in the earlier than the later months after the operation. Raw starch was not affected. He found that dextrose (not maltose) was formed both from starch and glycogen. No fat-splitting action was manifest, but the juice easily emulsified fat. The loop experimented upon was found to be situated about three times as far from the stomach as from the large intestine. Pregl calculates that the whole intestine would secrete nearly 3 litres in twenty-four hours.

It is difficult to say to what extent we are justified, from experiments performed on isolated loops, in forming conclusions regarding the nature of normal succus entericus. The first question that suggests itself is, How far is the fluid secreted a catarrhal production? As above stated, Pregl has pointed out that the mere prolapse of the gut causes a catarrhal increase above what he regards as the ordinary flow. The facility with which micro-organisms could enter would tend to increase any pathological condition. The presence of albumose in a fluid which does not digest proteid, and also of urea, suggests a pathological condition. Many of the ferment powers attributed to the juice might be due simply to desquamated epithelium from the walls of the loop.

Klecki[1] has criticised in the same way the experiments of L. Hermann, Blitstein and Ehrenthal, and Voit, dwelling on the abnormal conditions of the loop, and the small number of experiments upon which their conclusions are based. He himself finds that when few micro-organisms are allowed to remain in the gut, much less solid substance is finally found, and states that a large amount of contents is found in Hermann's rings only when the intestinal wall shows pathological changes, or if complete disinfection of the loop has not been carried out.

It would seem, therefore, that we must hesitate before accepting all the conclusions that have been drawn from the employment of the methods of isolated loops and Thiry-Vella fistulæ, bearing in mind that the juice so obtained is probably seldom entirely uninfluenced by the abnormal condition induced by the operation. Many, however, regard it as probable that the crypts of Lieberkühn, through their lining epithelium, yield a secretion which is of assistance in dissolving the products of digestion by other juices, even if it has no very well-marked digestive properties itself.

We may finally proceed to consider how far extracts made from the intestinal wall are characterised by the possession of specific properties.

In the first place, we must bear in mind that the intestinal mucous membrane has primarily, without doubt, an *absorbing* function. We have also reason to believe that the digested food in its passage through the epithelial cells may undergo considerable changes. Consequently, on making extracts of these epithelial cells, we may be separating substances which are never secreted into the lumen of the intestine, but which merely exercise influence on the absorbed food as it passes through the

[1] *Wien. klin. Wchnschr.*, 1894, Bd. vii.

cells. It is, therefore, not justifiable to assume that the secreted juice has the same action as an extract of the intestinal mucous membrane.

That extracts of the intestinal mucous membrane have marked physiological properties, there is little doubt. It is comparatively easy to make such extracts free from micro-organisms, and it is generally agreed that these extracts have a considerable power of inverting cane-sugar and of changing starch, in an intense degree, into dextrose, probably through the stage of maltose.

MECHANISM OF BILE SECRETION.

By D. Noël Paton.

Contents.—Mode of Formation of Bile Constituents, p. 559—Water, p. 559—Inorganic Salts, p. 560—Nucleo-Proteid, p. 561—Bile Acids, p. 562—Bile Pigments, p. 563—Cholesterin, p. 564—Lecithin, etc., p. 564—Influence of various Factors on the Secretion of Bile, p. 564—Flow of Blood, p. 565—Food, p. 565—Pressure of other Organs, p. 567—Nerves, p. 567—Chemical Substances, p. 567—General Conclusions, p. 569.

In considering the mechanism of bile secretion, it must be remembered that the formation of bile is only one of many functions performed by the liver.

Placed as it is upon the course of the portal vein, the great channel of absorption of material from the alimentary canal, the liver regulates the supply of carbohydrates to the body by storing the surplus sugar absorbed in the form of glycogen. It also gets rid of any excess of nitrogen absorbed, by converting it into the innocuous and easily eliminated urea. In addition to performing these functions, the liver acts as one of the great storehouses of iron in the body, and in many animals it is also a situation in which surplus fats are accumulated.

When these numerous functions are considered, the small amount of bile formed per diem by so large an organ is the less surprising. In man about 800 or 900 grms. of bile, with about 14 or 15 grms. of solids, are daily secreted from the liver, an organ which weighs about 1600 grms.

In studying how bile is formed in the liver, it is necessary to remember that, besides the great mass of liver cells, there are innumerable bile passages lined by a living epithelium. In most animals a saccular diverticulum, the gall bladder, is developed upon these passages. In this and in the passages the surplus bile accumulates. How far the liver cells, and how far the cells lining the ducts, act in producing the various constituents of bile, must be subsequently considered.

The bile is a fluid containing many different substances in solution (see article, "Chemistry of Bile"), and an investigation of the mechanism of bile secretion necessitates a consideration of the mode of production of each of these.

Mode of Formation of Bile Constituents.

Water.—The water of the bile is in part secreted from the walls of the bile passages, for it has been found that when the cystic duct is occluded, and the fundus of the gall bladder opened, a small amount

of fluid, about 70 c.c. per diem, is continually secreted from the walls of the gall bladder.[1] How far this fluid is a physiological secretion, and how far it is due to pathological conditions, is difficult to decide.

That water is secreted by the liver cells, as well as by the cells of the ducts, is proved by the way in which pigments,[2] which are secreted by the liver cells alone, are washed down into the bile passages.

The elimination of the water of the bile is a process of secretion, and not of transudation. Heidenhain's observations on the relative pressures in the bile passages and in the blood vessels passing to the liver,[3] given in the following table, demonstrate very clearly that, though the pressure of secretion of bile is low, it is nevertheless considerably higher than the blood pressure in the portal vein.

No.	Bile Pressure.	Pressure in Vena Mesenterica Superior.
1	220 mm. carbonate of soda sol.	90 mm. carbonate of soda sol.
2	175 ,, ,,	67 ,, ,,
3	204 ,, ,,	90 ,, ,,
4	110 ,, ,,	50 ,, ,,
5	180 ,, ,,	65 ,, ,,

The absorption of water from the alimentary canal seems under certain conditions to increase the secretion of water by the liver.

Rohrig,[4] Bidder and Schmidt,[5] and Zalesky,[6] noticed that the introduction of water into the stomach and intestine of dogs with biliary fistulæ increased the flow of bile. Rosenberg[7] found that if the intestine had previously been cleared out by a glycerin enema, the introduction of 500 c.c. of water into the intestine increased the flow of bile. In a case of complete biliary fistula in a woman,[8] the amount of the bile secretion was greater upon the days on which a large quantity of fluid was taken, and this increase was in the water of the bile, not in the solids.

Inorganic salts.—The analyses of the bile of the dog given by Hoppe-Seyler,[9] show that in bile taken from the gall bladder the salts constitute about 5 per cent. of the solids, while in freshly secreted bile they amount to about 13 or 14 per cent. The freshly secreted bile alone need be considered in discussing the mode of formation of these salts. A comparison of the salts of the bile with the salts of the blood plasma indicates that the percentage amount of salts is smaller in bile than in blood, and that, while chloride of sodium is the most abundant in

[1] Birch and Spong, *Journ. Physiol.*, Cambridge and London, vol. viii. p. 378 ; Mayo Robson, *Proc. Roy. Soc. London*, 1890, vol. xlvii. p. 499.

[2] Wertheimer, *Arch. de physiol. norm. et path.*, Paris, 1891, p. 724.

[3] Hermann's "Handbuch," Bd. v. S. 269.

[4] *Med. Jahrb.*, Wien, 1873, Bd. ii.

[5] "Die Verdauungssäfte," 1852, S. 166.

[6] Hofmann and Schwalbe, *Jahresb. ü. d. Fortschr. d. Anat. u. Physiol.*, Leipzig, 1877, S. 219.

[7] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 361.

[8] Noël Paton and Balfour, *Rep. Lab. Roy. Coll. Phys.*, Edin., 1891, vol. iii. p. 191.

[9] "Physiol. Chem.," S. 302.

both, in bile the proportion of this salt is not nearly so high as in plasma. This may possibly be explained by the withdrawal of hydrochloric acid in the stomach, leaving the soda to be combined with the organic acids of the bile.

A study of the excretion of chlorine in the bile has been made by Dagnini in Albertoni's laboratory.[1] He finds that in dogs with a permanent fistula the percentage of chlorine varies little, and that it is only slightly raised by the administration of chloride of sodium, or of potassium. Chlorides, as is well known, are chiefly excreted by the kidney.

Giovanni Pirri [2] has studied the secretion of sodium and potassium, and finds that, while the amount of sodium excreted per diem is very constant in spite of variations in diet, and in spite of the administration of chloride of sodium, the excretion of potassium varies within wide limits, and is increased by giving sodium and potassium chloride in the food. The sodium is in great measure combined with the organic acids of the bile, and hence these results do not throw light upon the excretion of sodium in inorganic compounds.

On the secretion of lime salts, work has been done under Naunyn's direction by Jankau. He shows that the amount of lime in bile is very small, and that it is not increased by the administration of lime salts.[3] From the fact that lime salts are present in the secretions from mucous membranes, Naunyn suggests that the lime of the bile may be formed in the bile passages.

The very small quantity of iron which exists in the bile (less than 1 mgr. per diem in the dog)[4] may be derived from the iron stored in the liver cells, or may be formed from the disintegration of the epithelial lining of the passages. Evidence on the subject is wanting.

How far the other inorganic salts are secreted by the liver cells, and how far by the cells lining the bile passages, cannot be considered as established. There is clear evidence to show that they are, in part at any rate, formed in the latter situation. In a series of analyses of bile, collected from a woman with a complete biliary fistula, it was found that during attacks of fever the true biliary constituents, the organic salts and pigments, were markedly diminished, while the proportion of inorganic salts remained unaltered, between 0·7 and 0·8 per cent.[5]

Birch and Spong's analysis of the fluid from the gall bladder showed the presence of 0·826 per cent. of inorganic salts, of which the chief was chloride of sodium. Mayo Robson found 0·84 per cent. of inorganic matter. Analysis of freshly secreted human bile gives about the same proportion of salts.[6] Hence, since the amount of salts is the same in the small amount of fluid secreted from the bile passages, and in the total amount of bile poured out from bile passages and liver cells together, about the same proportion of salts must exist in the secretion from each.

Nucleo-proteid.—The mucus-like nucleo-proteid of bile is formed in the bile passages and gall bladder. The amount in bile is small, about 0·2 per cent.

[1] *Mem. r. Accad. d. sc. d. Ist. di Bologna*, 1893, Ser. 5, vol. i. p. 3.
[2] *Ibid.*, 1893.
[3] Naunyn, "Cholelithiasis," translated by A. E. Garrod, *New. Syd. Soc.*, p. 15.
[4] Anselm, *Arb. d. pharmakol. Inst. zu Dorpat*, Stuttgart, 1892, Bd. vii.
[5] *Rep. Lab. Roy. Coll. Phys.*, Edin., vol. iv. p. 44.
[6] Hoppe-Seyler, "Physiol. Chem.," S. 302.

In cases of occluded gall bladder this mucin-like substance has been found to be the chief organic solid of the secretion.[1]

Mayo Robson's Analysis.

Organic matter, chiefly mucin . . .	·672 per cent.
Chlorides equal to NaCl	·573 „
Sodium carbonate	·220 „
Other salts containing phosphates, potassium salts, etc.	·071 „

The fact that the amount of this substance does not vary with the true bile constituents either at different periods of the day,[2] or in febrile conditions,[3] indicates very clearly that it is not formed by the liver cells.

Salts of the bile acids.—These are entirely produced in the liver cells. In Birch and Spong's case, and in the case examined by Mayo Robson, they were entirely absent from the secretion of the gall bladder.

That they are actually formed by the liver cells, and not merely extracted from the blood, was demonstrated by Minkowski and Naunyn.[4] These observers found that, while bile salts are normally absent from the blood, they appear when the bile duct is ligatured. If, however, the liver be excluded from the circulation, there is no accumulation of bile salts in the blood.

The source of the cholalic acid moiety of the glycocholic and taurocholic acids is unknown. The source of the glycine and taurine is to be sought ultimately in the proteids of the body and of the food, since these alone can yield the nitrogen and sulphur. Both are amido-acids of the fatty acid series.

Nencki, Pawlow and Zaleski,[5] have shown that the surplus proteid of the diet is largely broken down into ammonia compounds in the wall of the intestine, and these compounds pass to the liver. Von Schröder[6] demonstrated that ammonia compounds are readily converted to urea by the liver. Hence by far the greater quantity of nitrogen in excess of that required must undergo this transformation, and it is not to be expected that an additional quantity of proteids in the food will lead to a markedly increased formation of bile acids. Spiro,[7] by feeding animals with biliary fistulæ upon various kinds of food, found that a proteid diet increased the nitrogen and sulphur excreted in the bile, but not in proportion to the amount of proteid taken.

The following figures illustrate Spiro's results:—

Food.	Sulphur of Bile in Grms.	Nitrogen of Bile in Grms.
Fasting	·059	·195
125 grms. flesh . . .	·089	·292
500 „ . . .	·155	·398
949 „ . . .	·173	·604

[1] Hoppe-Seyler, "Physiol. Chem.," S. 302.
[2] *Rep. Lab. Roy. Coll. Phys.*, Edinburgh, vol. iii. p. 204.
[3] *Ibid.* p. 212. [4] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxi. S. 7.
[5] *Ibid.*, Bd. xxxvi. S. 26.
[6] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. ii. S. 234.
[7] *Arch. f. Physiol.*, Leipzig, 1880, Supp. Bd. S. 50.

Kunkel,[1] from similar experiments on dogs, concluded that a definite part of the sulphur taken in the diet is excreted in the bile, but the increase in biliary sulphur occurs two or three days after the ingestion, and not upon the same day, as is the case with the sulphur of the urine. Of the sulphur of the food, from 8 to 30 per cent. is excreted in taurine.

In man on an ordinary diet, about 33 grms. of urea with 15 grms. of nitrogen are daily formed, while only about 10 grms. of bile acids with about 0·3 grms. of nitrogen are excreted. The increased ingestion of proteids leads to a proportionate increased excretion of urea, and any increase in the bile acids is necessarily so small that it may readily be overlooked. Similarly, any increased decomposition of the proteids of the tissues leads to a proportionately increased excretion of the nitrogen in the form of urea, and any increase in the bile acids which may occur must be very trifling.

Whether the bile acids which are absorbed from the intestine can be again excreted by the liver cells, has been investigated by injecting into the blood of animals a bile salt differing from that which is normally present. In dogs the taurocholate of soda is the normal salt of the bile. After injecting glycocholate of soda, Prévost and Binet,[2] and Weiss[3] found it in the dog's bile. Socoloff,[4] on the other hand, failed to detect it after it had been injected. Huppert[5] observed that the injection of glycocholic acid increases the amount of bile acids in the bile. The experiments of Rosenberg[6] show that the administration of bile salts causes an increased secretion of bile with a marked increase in the solids. They appear to be the only substances which produce this result, and since the bile salts are the most abundant solids of bile, it seems fairly certain that they are absorbed, and re-excreted from the blood by the liver.

Bile pigments.—The pigments must be produced in the liver cells, since the secretion from the bile passages is entirely destitute of colouring matter.[7] They are formed from the hæmatin moiety of the hæmoglobin molecule. The injection of free hæmoglobin into the blood,[8] or the setting free of hæmoglobin by solution of the red corpuscles,[9] rapidly leads to a great increase of the bilirubin of the bile. Minkowski and Naunyn, by experiments upon birds,[10] have confirmed these observations. They further found that if the liver is excluded from the circulation the formation of bilirubin does not take place. They thus showed that bilirubin is actually produced in the liver cells. The iron-containing part of the hæmatin molecule appears to be split off and retained in these cells, giving rise to the accumulation of iron in the liver, which follows the disintegration of red corpuscles.[11]

Not only do the liver cells manufacture bilirubin, but when this or any other bile pigment is present in the blood they take it up and eliminate

[1] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xiv. S. 344.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvi. p. 1690.
[3] *Bull. Soc. imp. d. nat. de Moscou*, 1884.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. xi. S. 166.
[5] *Arch. d. Heilk.*, Leipzig, 1869, Bd. v.
[6] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 334.
[7] Birch and Spong, *Journ. Physiol.*, Cambridge and London, vol. viii. p. 378; Mayo Robson, *Proc. Roy. Soc. London*, 1890, vol. xlvii. p. 499.
[8] Städelmann, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1890, Bd. xvii. S. 93.
[9] Afanassiew, *Ztschr. f. klin. Med.*, Berlin, Bd. vi. Heft 4.
[10] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xxi. S. 1.
[11] Hunter, *Lancet*, London, 1892, p. 1262.

it. This was definitely proved by Wertheimer,[1] who injected into the circulation of dogs the bile of the ox and sheep. The bile of these animals contains a pigment, cholohæmatin, which gives a characteristic spectrum, and the appearance of this spectrum in the bile of the dog showed that cholohæmatin had been taken up and excreted.

Cholesterin.—Whether cholesterin is formed in the liver cells, or in the cells lining the bile passages, or in both, is not definitely known. In the two cases of fistula of the gall bladder already referred to, the presence or absence of cholesterin is not noted.[2]

That the cholesterin is formed somewhere within the liver, and not merely excreted by it, is shown by an experiment by Jankau, performed in Naunyn's laboratory.[3] He injected cholesterin into dogs, and also gave it in their food, and ascertained that it had been absorbed; but he failed to find any increase of cholesterin in the liver tissue, or in the bile. The analyses of the liver and bile made by Kausch[4] in the same laboratory show no relationship between the amount of cholesterin in the gland, and in its secretion. Thomas,[3] who also worked in Naunyn's laboratory, found that there is no relationship between the amount of cholesterin excreted and the kind of food taken. When the dog under observation suffered from catarrh of the biliary passages, there was a marked increase in the cholesterin of the bile.

From these experiments, and from the fact that cholesterin is always found where cells are disintegrating, Naunyn strongly supports the view that cholesterin is produced, not in the liver cells, but from the cells of the passages, and that it is a product of the disintegration of their protoplasm.

Lecithin and other compounds of the fatty acids.—The occurrence of these bodies in the secretion from the gall bladder has not been observed. On the other hand, lecithin and fat are constant and abundant constituents of liver cells. Liver tissue contains about 2·35 per cent. of lecithin, and about 3 or 4 per cent. of fat.[5] Thomas found[6] that, while cholesterin was unaltered in amount by the administration of various diets, the amount of fat in the bile depended largely upon the amount of fat taken in the food; and since the fats of the food are frequently stored in the liver cells, it is probable that the fatty acid compounds in the bile are derived from this source.

Influence of Various Factors upon the Secretion.

The investigation of the influence of varying conditions upon bile secretion is a matter of extreme difficulty, for the bile may accumulate in the gall bladder and passages to be expelled from the liver some time after secretion.

The flow of bile is governed by—

1. The rate of secretion.
2. The activity of the muscular walls of the passages.
3. The pressure upon the liver of adjacent organs.

[1] *Arch. de physiol. norm. et path.*, Paris, 1891, p. 724.

[2] In the colourless fluid from a case of hydrops cystidis felleæ, I found a considerable quantity of cholesterin.

[3] "Cholelithiasis," translated by A. E. Garrod, *New Syd. Soc.*, 1896.

[4] Diss., Strassburg, 1891.

[5] Noël Paton, *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. 213.

[6] *Loc. cit.*

Further, the liver being placed upon the efferent vessel of the alimentary canal, must have its vascular condition altered by every modification in that of the gastro-intestinal tract, and it is impossible to eliminate this element while studying the action of any agency on bile secretion.

Influence of the hepatic circulation upon bile secretion.—The circulation in the liver may be profoundly altered without actual stoppage of bile secretion. Thus it has been shown, in cases where, by the method devised by Oré, the portal blood has been directed into the inferior vena cava, that bile is still secreted by the liver;[1] while Wertheimer[2] has confirmed the results of older investigators, that ligature of the hepatic artery does not immediately stop the secretion, although ultimately necrosis of liver tissue supervenes and leads to abolition of function.

But while these marked disturbances do not at once stop secretion, there is evidence that its rate depends upon the vascular supply. Thus Heidenhain has shown[3] that in dogs, section of the splanchnic nerves, which causes a dilatation of the portal vessels, produces a marked increase in the flow of bile. If, however, this local dilatation is accompanied by a general dilatation, such as is produced by section of the spinal cord in the neck, a fall in the secretion occurs. Munk,[4] on the other hand, has shown that stimulation of the splanchnic nerves, which produces constriction of the vessels, leads to a diminution in the rate of bile secretion.

How far this influence of alteration in the blood supply is due to variation in pressure, and how far to alteration in the rate of blood flow through the liver, has not been directly investigated. But the observation of Röhrig,[5] that constriction of the vena cava inferior, which raises the pressure in the liver while decreasing the rate of blood flow, diminishes bile secretion, seems to indicate that the rate of flow is of more importance than the mere intravascular pressure. In this connection the relationship of the pressure of secretion to blood pressure (p. 560) must be borne in mind.

Effects of food.—Starvation, according to Bidder and Schmidt, causes a diminution in the amount of bile secretion, and a corresponding fall in the amount of solids.[6] Their experiments are unsatisfactory, in so far that cats were taken at various stages of starvation up to 240 hours after food, a temporary fistula made, and the bile secretion determined for a short period only. The most recent contribution to our knowledge of this subject was made by Lukjanow,[7] who determined the changes in the various solids of the bile in guinea-pigs kept without food or water. He concludes that both the secretion of water and of solids diminishes throughout the period of fasting.

From the investigations on the relationship of bile secretion to the flow of blood through the liver, it is obvious that the dilatation of the abdominal vessels, which occurs in digestion, will of itself cause an increased secretion of bile. Such an increased flow has

[1] *Arch. d. sc. biol.*, St. Pétersbourg, 1892, vol. ii.
[2] *Arch. de physiol. norm. et path.*, Paris, 1892, p. 577.
[3] Hermann's "Handbuch," Bd. v. S. 266.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. viii. S. 151.
[5] *Med. Jahrb.*, Wien, 1873, Bd. ii.
[6] Bidder and Schmidt, "Die Verdauungssäfte."
[7] *Ztschr. f. physiol. Chem.*, Strassburg, 1892, Bd. xvi. S. 87.

been observed by various investigators. The most careful observations on the influence of food in bile secretion are those recorded by Hoppe-Seyler.[1] The experiments were made on a dog with a permanent biliary fistula, and they show that within an hour after food the flow of bile is slightly and temporarily increased. It is very probable that this initial increase is simply due to reflex stimulation of the gall bladder and bile passages, expelling the bile already secreted. Four or five hours after a meal the flow is enormously increased, the amount of bile solids rising with the amount of bile. The extent of this accelerated flow indicates that it is actually an increased secretion. How far it is due to the increased vascularity of the abdominal viscera, and how far to the stimulating action of absorbed material on the liver cells, is not made manifest by the experiments. About nine or ten hours after a meal there is a secondary increase, not so marked as the first, but lasting for two or three hours, and accompanied by a still more marked rise in the excretion of solids. The cause of this is unknown.

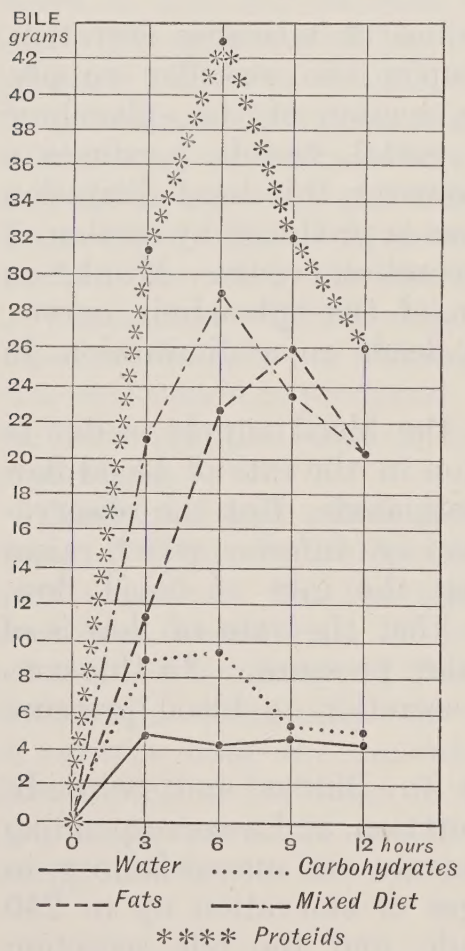


FIG. 48.—Showing influence of various food stuffs upon the secretion of bile.

As to the special influence of the various constituents of the food, our knowledge is somewhat defective. The researches of Rosenberg[2] and of Barbera,[3] on dogs with a permanent fistula, show an increase in the secretion of bile and of the bile solids after proteid food. The latter observer states that carbohydrates have also a certain effect in increasing the secretion of bile, but that their effect is very small indeed. Both observers find that the administration of fats very markedly increases the bile flow; but while in Rosenberg's experiments the flow of bile under the influence of fats was greater than with proteids, in Barbera's the increase was most marked on a diet of flesh.

The accompanying chart (Fig. 48) gives a summary of Barbera's observations.

In another paper Barbera[4] shows that the excretion of bile after a meal of proteids or carbohydrates runs parallel with the secretion of urea, but that after a meal of fats the bile secretion increases out of proportion to the urea.

The slight increase in the secretion following the administration of carbohydrates is probably due to the vascular dilatation. The more marked increase after fats may be related to their more prolonged digestion, and the correspondingly greater and more sustained dilatation of vessels. The increase after proteids is in part due to the same cause, but may also be due to the increased functional activity of the liver,

[1] "Physiol. Chem.," S. 308.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 243.
[3] *Bull. d. sc. med. di Bologna*, 1894, Ser. 7, vol. v.
[4] "Rapporto tra la eleminazione dell urea e della bile."

which has to deal with nitrogen in excess of the requirements of the body.

After every kind of food the absorption of bile salts and their action on the liver must be taken into account as a factor in increasing the flow of bile (see p. 563).

Influence of pressure of surrounding structures.—The liver, being situated just below the diaphragm and above the abdominal viscera, is subject to marked variations in pressure. It has already been pointed out that a considerable quantity of bile may collect in the bile passages. By pressure from adjacent organs, this may be squeezed out. The facts that section of one vagus reduces the bile flow only when the frequency of respiration is diminished,[1] and that section of the vagus just above the diaphragm, which has no influence on the rate of respiration, leaves the bile secretion unaltered, and that stimulation has also no effect, seem to indicate that the flow of bile is accelerated by respiratory movements.

The very marked rise in the amount of bile poured out between four and eight A.M. in a case of biliary fistula,[2] just at the time when the patient wakened and commenced to move about, further supports the view that pressure on the liver may cause an increased flow of bile.

Direct influence of nerves upon bile secretion.—It has already been pointed out that the secretion of bile may be indirectly modified by the influence of nerves upon the blood vessels. The flow of bile may also be increased through the stimulation of the nerves to the muscular coat of the bile ducts and gall bladder. Reflex stimulation through these nerves probably accounts for the first gush of bile after food is taken. There is, however, no evidence that stimulation of nerves can directly increase or diminish the actual secretion of bile—any change in the flow being fully explained by indirect action. The facts that the injection of pilocarpine, which so markedly increases the flow of saliva and of pancreatic juice, has no influence on bile secretion,[3] and that atropine has no action in arresting the secretion,[4] seem to oppose the idea that there is any direct nervous influence upon the process.

Influence of various chemical substances on bile secretion.—Certain substances, when introduced into the portal blood, either directly or through the alimentary canal, cause an increase in the secretion of bile.

Tarchanoff[5] found that when hæmoglobin is injected into the blood-vessels the bilirubin of the bile is increased in amount. Städelman[6] and Afanassiew[7] afterwards demonstrated that such drugs as toluylenediamin and arseniuretted hydrogen, which cause the solution of hæmoglobin from the blood corpuscles, produce not only an increase in the bilirubin of bile, but also an increased flow of bile, and that this polycholia seems to be proportionate to the destruction of hæmoglobin. It is therefore clear that the passage of free hæmoglobin to the liver acts as a stimulant, and may produce an increased flow of bile; and hence all substances which bring about an escape of the blood colouring-matter tend to increase the secretion of bile.

[1] Hermann's "Handbuch," S. 270.
[2] *Rep. Lab. Roy. Coll. Phys.*, Edinburgh, vol. iii. p. 200.
[3] Paschkis, *Med. Jahrb.*, Wien, 1884, S. 169.
[4] Rutherford, "Action of Drugs on the Secretion of Bile," Edinburgh, 1880, p. 96.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. ix.
[6] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1883, Bd. xcviii. S. 460.
[7] *Virchow's Archiv*, 1884, Bd. xcviii. S. 460.

Among these substances are the salts of the bile acids, and all investigators find that the administration of these causes an enormous increase in bile secretion. But while such pure hæmolytics as toluylenediamin and arseniuretted hydrogen cause only a transitory increase in the secretion, and produce a very concentrated bile, the bile salts not only markedly increase the solids, but also the water secreted. The following record of one of Rosenberg's [1] experiments shows this effect:—

Time in Hours.	Amount of Bile.	Per Cent. of Water.	Per Cent. of Solids.
At 8.30–9.30 A.M. . .	4·7673	94·4	5·6
	10 grs. bile with 1·16 grs. solids given at 9.30		
,, 9.30–10.30 ,, . .	6·9095	95·3	4·7
,, 10.30–11.30 ,, . .	12·9783	93·9	6·1
,, 11.30 A.M.–12.30 P.M. .	3·6582	90·8	9·2
,, 12.30–1.30 P.M. . .	2·3897	88·9	11·1

Again, Städelman's work shows that, while the ordinary hæmolytics do not increase the secretion of bile salts, the administration of the bile salts leads to a marked increase in their percentage amount in the bile. Paschkis' experiments [2] indicate that, while glycine and taurine have little action as cholagogues, cholalic acid is exceedingly active. It would thus seem that these substances act not only in virtue of their hæmolytic action, but by reason of a special stimulating influence upon the liver cells.

Salicylate of soda, which also has a hæmolytic action, greatly increases the flow of bile. But while the bile salts cause an increase in the solids, this substance produces a very marked dilution of the bile (Rutherford,[3] Lewaschew,[4] and Rosenberg [5]).

One of Rosenberg's experiments is here given to show this effect.

Time in Hours.	Bile Secreted.	Per Cent. of Water.	Per Cent. of Solids.
At 8–9 A.M. . . .	1·2944	80·6	19·3
,, 9 ,, . . .	2·0 grs. salicylate of soda given		
,, 9–10 ,, . . .	6·2885	90·7	9·3
,, 10–11 ,, . . .	4·2914	92·3	7·7
,, 11 A.M.–12 noon .	4·5218	92·1	7·9
,, 12 noon–1 P.M. . .	4·6437	91·8	8·2

[1] *Loc. cit.* [2] *Loc. cit.*
[3] "Action of Drugs on the Secretion of Bile," Edinburgh, 1880, p. 118.
[4] *Ztschr. f. klin. Med.*, Berlin, 1884, Bd. viii. S. 67.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 355.

Rutherford, Vignal, and Dodds have experimented with a very large number of drugs, which were injected, dissolved in bile, into the duodenum.[1] The action of certain of these drugs has been re-investigated by Paschkis [2] and by Lewaschew,[3] whose results do not in all cases confirm those of the previous observers. It is, however, unnecessary to consider them in detail. Naunyn [4] sums up the matter by saying, "Many substances, when taken into the stomach, and more surely still when introduced into the duodenum (Rutherford), appear to produce under certain conditions a slight increase of the biliary secretion. But the influence of these substances upon the secretion of bile is uncertain, and never a potent one."

General Conclusions.

From a study of the mechanism of bile secretion, it is manifest that in its bile-producing function the liver differs from most other glands, since its activity is not under the direct control of the nervous system, but is modified by the ebb and flow of the blood stream, and by the influence of various chemical substances, such as the salts of the bile acids.

The relationship of bile secretion to the other functions of the liver is in many points still obscure. That the disintegration of hæmoglobin and the formation of bile pigments are closely connected, is definitely known (p. 563). That these two functions are connected with the production of urea, is shown by the fact that the administration of hæmolytic agents, such as toluylenediamin, pyrogallic acid, etc., which increase the formation of bilirubin, cause a proportionate increase in the disintegration of red blood corpuscles, and in the excretion of urea.[5]

How far the formation of the amido-acids of the bile salts is connected with the disintegration of proteids, cannot be considered as settled, but the evidence adduced on p. 562 suggests that such a relationship exists. If this be the case, the formation of biliary constituents must be connected with the manufacture of glycogen and glucose from proteids. The formation of bile seems independent of the mere accumulation of carbohydrates in the liver.

The various compounds of fatty acids in the bile are probably derived from the fatty acid compounds stored in the liver (p. 564). The nucleo-proteid, the mucin, and the cholesterin are probably to be regarded, not as true biliary constituents, but as products of the bile passages. As to the relationship of the inorganic salts of the bile with the other hepatic functions, nothing is known.

[1] Rutherford, *loc. cit.* [2] *Loc. cit.* [3] *Loc. cit.*
[4] "Cholelithiasis," translated by A. E. Garrod, *New Syd. Soc.*, p. 172.
[5] Noël Paton, *Brit. Med. Journ.*, London, 1886, vol. ii. p. 207.

THE CHEMISTRY OF THE URINE.

By F. Gowland Hopkins.

Contents :—Introductory—Quantitative Composition of Urine, p. 572—Variations in its Amount and Specific Gravity, p. 573—Its Chemical Reaction, p. 574—The Nitrogenous Constituents : (*a*) Total Nitrogen, p. 580 ; (*b*) Urea, p. 581 ; (*c*) Ammonia, p. 585 ; (*d*) Uric Acid, p. 586 ; (*e*) Xanthin Bases, p. 596 ; (*f*) Creatinin, p. 598 ; (*g*) Hippuric Acid, p. 600 ; (*h*) Amido-Acids, p. 602—Proteids, p. 603—The Aromatic Substances, p. 605—The Carbohydrates, p. 607—Glycuronic Acid and its Conjugated Compounds, p. 613—Oxalic Acid, p. 614—Acids and Oxyacids of the Fatty Series, p. 615—Colour of the Urine and the Chemistry of its Pigments, p. 616 : (*a*) The Preformed Pigments of Normal Urine, p. 618 ; (*b*) Chromogenic Substances, p. 626 ; (*c*) The Pigmentation of Pathological Urine, p. 628—The Inorganic Constituents, p. 630—General Characteristics of the Organic Urinary Compounds, p. 635—Comparative Chemistry of the Urine, p. 637.

General considerations.—The chemical study of the urine gains its chief importance from the light which it throws upon the processes of metabolism. It is concerned mainly with a consideration of the nature and amount of the various metabolic end-products, normal or pathological, which converge into and appear together in the highly complex excretion of the kidneys.

The great importance of this point of view has led to perhaps undue neglect of a second aspect of the subject—the consideration of the renal excretion as a complex whole ; as a chemical fluid with individual characters of its own ; characters which are not to be foretold from a knowledge of the nature and amount of each constituent considered separately, but require for their explanation the further consideration of the mutual effects of the constituents one upon another, as they exist side by side in solution.

This study of the properties of the urine as a whole must be pursued if we are to understand with exactness the nature of the processes which go on in the kidney, and if we wish to interpret aright the ultimate behaviour of any given type of urine while in the urinary passages, or after it has left the body.

But while the first-mentioned line of study requires in the main the services only of analysis—the earliest and best understood of the weapons of chemistry—the second depends upon our more recently won, and as yet very incomplete, knowledge of chemical statics, and of the conditions of equilibrium in salt solutions.

All the chief proximate constituents of normal urine exhibit either basic or acid characters. Indifferent or "neutral" substances are normally either absent, or present in minimal amount. The bases and acids present necessarily enter into more or less stable combinations, and it

follows that the urine is essentially a solution of salts; its chemical and physical properties being those of a complex saline mixture.

The chief bases are potassium, sodium, and ammonium; calcium and magnesium; urea, creatinin, and the xanthin bases. The chief acids are hydrochloric and sulphuric; phosphoric and carbonic; uric; oxalic; with hippuric and certain other aromatic acids. To the acid group belong also undoubtedly the pigments.

The particular combinations formed in the urine by these various acids and bases depend primarily on their relative masses and avidities; the ultimate equilibrium of the fluid depending, secondarily, on the mutual influences, in solution, of the salts which potentially tend to form as a result of the two factors just mentioned. It should be understood that our present knowledge does not carry us far towards a calculation of this complex equilibrium in any particular case. When we have determined by analysis the proportions of the various bases and acids present, we may, for convenience, group them into various supposititious combinations one with another, and speak of the urine as containing so much sodium chloride, so much "earthy phosphates," and the like; but such groupings can, with our present knowledge, be for the most part approximate only; and, if insisted upon too closely, may be misleading.

If the chemistry of urine had to be read merely as a final chapter in the history of metabolism, the actual condition of the acids and bases present would be of little importance to the physiologist or to the pathologist. The nature and amount of these constituents having been determined, each would be considered in connection with the organ or tissue the metabolism of which is responsible for its appearance in the urine, and the chemistry of the latter would be of no further import.

But the case, as we have said, is otherwise. The two conditions of chemical equilibrium represented respectively by the expressions—

(1) $CaSO_4 + 2(NaH_2PO_4)$ [three molecules]
(2) $Na_2SO_4 + Ca(H_2PO_4)_2$ [two molecules]

involve each of them the same amount of the bases and acids concerned; but the presence of the first combination in the urine might involve a renal activity quantitatively as well as qualitatively different from that which would be indicated by the presence of the latter. Further, a knowledge merely of the percentage of uric acid in a given specimen of urine will by no means give us final information as to the power of the fluid to retain this constituent in solution. One individual may excrete a large percentage, and yet have no tendency to suffer from uric acid gravel; another may not be free from this, though he habitually excrete a lower percentage. To explain this we must understand the influence of other urinary constituents on the solubility of uric acid; in other words, we must study the properties of the urine as a whole.

Enough has been said to show that we are not to remain content with analytical figures alone. The future study of the urine will concern itself also with the application of facts derived from that domain of chemistry which deals with the distribution of chemical forces in complex mixtures. At present we have but little available knowledge of this kind, and many urinary phenomena are consequently but imperfectly understood. We may instance, however, a generalisation made from the experimental and mathematical investigation of the mutual influence of salts in solution, which is capable of immediate application to our subject.

If two salts contain an electrical *ion* in common (or without great inaccuracy we may say, a base or acid in common), each *decreases* the solubility of the other, whereas salts which contain no base or acid in common may mutually *increase* each the other's solubility. Thus the presence of sodium chloride in solution will diminish the solubility of sodium urate,[1] and ammonium chloride that of ammonium urate; but the presence of either of these chlorides will increase the solubility of (say) calcium phosphate. These laws will be found to have important application in the explanation of certain urinary phenomena.

In addition to products which arise from metabolism in the tissues, the urine contains substances which are derived more directly from the ingesta. These comprise a large proportion of the normal inorganic constituents, which are always found in the diet in excess of the needs of the organism; and they may consist also of substances accidental or accessory to the diet, or again of drugs, or of substances experimentally introduced into the body.

Some of these, while taking no share in metabolism proper, may form "conjugated" or synthetic compounds with certain intermediate products of metabolism, and so modify excretion. Thus glycin and glycuronic acid are substances capable of easy oxidation in the body, and are therefore not properly terminal products of metabolism; but they are protected from oxidation and are eliminated as synthetic compounds with certain aromatic substances, whenever the latter are absorbed in sufficient quantity from the bowel.

Quantitative Composition of the Urine.

The figures which follow are from the well-known table given by Parkes, representing the normal twenty-four hours' excretion of the chief urinary constituents:—

	Percentage Composition of Solids.	Absolute Weight of Solids in Grms.	Weight per 1000 of Body-Weight.
Urea, CH_4N_2O	45·75	33·18	0·5000
Creatinine, $C_4H_7N_3O$	1·25	0·91	0·0140
Uric acid, $C_5H_4N_4O_3$	0·75	0·55	0·0084
Hippuric acid, $C_9H_9NO_3$	0·55	0·40	0·0060
Pigment and other organic substances	13·79	10·00	0·1510
Sulphuric acid, SO_3	2·77	2·01	0·0305
Phosphoric acid, P_2O_5	4·36	3·16	0·0480
Calcium	0·35	0·26	0·0004
Magnesium	0·28	0·21	0·0003
Potassium	3·45	2·50	0·0420
Sodium	15·29	11·09	0·1661
Chlorine	10·35	7·50	0·1260
Ammonia	1·06	0·77	0·0130
	100·00	72·54	1·1057

In the following analyses, derived from Bunge, all the figures were obtained from the same individual. They represent the twenty-four hours' excretion of a young man; in the one case, upon a diet consisting entirely of beef with a little salt and spring water; in the

[1] As was shown experimentally by Sir William Roberts, before the general principle enunciated above had been developed by Nernst.

other case, upon a diet of bread with a little butter, again with water as a beverage:—

	Meat Diet.	Bread Diet.
Total measure of urine in twenty-four hours	1672 c.c.	1920 c.c.
Urea	67·2 grms.	20·6 grms.
Creatinine	2·163 ,,	0·961 ,,
Uric acid	1·398 ,,	0·253 ,,
Sulphuric acid (total)	4·674 ,,	1·265 ,,
Phosphoric acid	3·437 ,,	1·658 ,,
Lime	0·328 ,,	0·339 ,,
Magnesia	0·294 ,,	0·139 ,,
Potash	3·308 ,,	1·314 ,,
Soda	3·991 ,,	3·923 ,,
Chlorine	3·817 ,,	4·996 ,,

These analyses are interesting as showing the effect of two widely differing forms of diet; but they must not be taken as typical of the relative effect of animal and vegetable diet in any absolute sense. As regards such factors, for instance, as the relative proportion between urea and uric acid, we shall find that, even when one or other of the two types of diet (animal or vegetable) is adhered to, great differences may be seen as the effect of variation in the specific composition of either. Indeed, no great importance must be attached to the details of collective quantitative analyses of the urine, except where the diet itself has been simultaneously analysed. While abundant observations of this kind have been published, relating to particular constituents of the urine, no collective analyses appear to have been made upon the same specimen of urine after a diet of known quantity and composition.

The following figures, which give the mean of many determinations made by Yvon and Berlioz, show the differences in the excretion of certain constituents by males and females respectively:—

	Male.		Female.	
	Per Litre.	Per Diem.	Per Litre.	Per Diem.
Specific gravity	...	1·0225	...	1·0215
Volume	...	1360 c.c.	...	1100 c.c.
Urea	21·5 grms.	26·5 grms.	19·0 grms.	20·5 grms.
Uric acid	0·5 ,,	0·6 ,,	0·55 ,,	0·57 ,,
Phosphoric acid	2·5 ,,	3·4 ,,	2·4 ,,	2·6 ,,

The Quantity of Urine and its Specific Gravity.

A human adult excretes from 1200 to 1700 c.c. of urine in the twenty-four hours, or about 1 c.c. per kilo. of body weight per hour. During sleep the amount is less than at other times. The specific gravity commonly varies from 1015 to 1025, and is, in general, inversely as the quantity excreted.

Both factors, however, may vary through much wider limits than those given, without any departure from conditions of health. The chief causes which lead to increase of quantity and diminution of density are increased consumption of liquid and diminished activity of the sweat-glands. With abstention from liquids, or increased activity of the skin, the amount necessarily falls, and the density is raised.

Increase in the quantity may follow, not alone from a heightened quantity of water in the blood, but from any influence, normal or pathological, which increases the blood flow through the kidneys.

Pathologically the quantity is increased in diabetes mellitus and insipidus, in certain stages of chronic nephritis, and in some neurotic conditions; it is decreased in the early stages of acute nephritis, in the congestive condition of cardiac disease, and when large quantities of fluid are lost by the bowel, as in cholera. The specific gravity is increased in diabetes, and diminished in chronic nephritis.

The specific gravity is roughly an indication of the amount of the urinary solids. It cannot indicate the amount with exactness, as the substances in solution are of various physical properties, and are not all capable of increasing the density in like proportions. Thus, while a 10 per cent. solution of common salt has (at 15°) a specific gravity of about 1073, a 10 per cent. solution of urea indicates only 1028.[1] An increase in the urinary salines would therefore have a much greater effect in raising the specific gravity than a like increase in the urea. A knowledge of the actual weight of solids present seldom becomes of much importance. It may be obtained with sufficient accuracy by multiplying the last two figures of the sp. gr. by 2·2; the result indicating the total solid matter in grammes per litre. Thus a specimen of sp. gr. 1020 contains about 44 grms. per litre of substances in solution.

Chemical Reaction.

Acids and bases are so proportioned in human urine that the mixed excretion of twenty-four hours generally reacts acid to litmus paper. It may sometimes exhibit the so-called amphoteric reaction—a phenomenon to be later discussed—but under strictly normal circumstances the accumulated excretion of the day is never alkaline to litmus. On the other hand, during limited periods of the daily cycle, it may sometimes, though not commonly, become alkaline.

Litmus is reddened both by acids and by acid salts; but there are other coloured indicators which behave differently in the presence of free acids and acid salts respectively. When such are applied to urine, they show unequivocally that the former are never present, and we are thus forced to the conclusion that urine owes its acidity to acid salts. It will be shown immediately that we may conclude with some certainty that the reaction is due, as a matter of fact, to the presence of acid phosphates.

The nitrogen, carbon, phosphorus, and sulphur of food-stuffs are all capable of oxidation to acid anhydrides, and the last three elements are in fact oxidised to this acidic form in the body. The chief product of the oxidation of carbon, carbon dioxide, may play a not unimportant rôle in the equilibrium of urinary acids and bases, and the existence of oxidised carbon in the molecules of certain organic compounds in the

[1] A. H. Allen, "Chemistry of Urine," 1895, p. 12.

urine confers upon them a definite acidic character. The acid oxides of phosphorus and sulphur, which are the chief end-products of the metabolism of these two elements, are eliminated almost entirely through the kidneys. Eighty per cent. of the total sulphur ingested, and nearly all the phosphorus, are eventually found in the urine as sulphuric and phosphoric acids respectively. That these acids are eliminated as salts, and not in the free state, depends in the main upon the fact that bases are continually being ingested in the food in a form available for the neutralisation of acids. The bases of the food are not all in the state of stable neutral salts. Even animal food contains basic phosphates, together with organic (proteid) combinations of the alkalies and alkaline earths, and small quantities of alkaline carbonates; while vegetable food contains, in addition, salts of the vegetable acids, which in the body are converted into carbonates by oxidation. By the ingestion of these unstable compounds of various bases, the organism is saved from the necessity of eliminating free mineral acids. When the supply of available bases is for any reason insufficient, a further protective mechanism comes into action, metabolism being so modified that a greater proportion of the nitrogen than usual is eliminated in the strongly basic form of ammonia. All these factors are normally so proportioned that, as we have seen, the urine, while containing no free acid, is acid from acid salts.

Phosphoric acid (H_3PO_4) as a tribasic acid forms three orders of salts. Those in which two out of the three hydrogen atoms of the acid molecule are intact, are known as acid or superphosphates. They are soluble salts, and react acid to litmus. The second type, in which two hydrogen atoms are replaced by a base (monohydrogen phosphates), and the third, in which all the hydrogen is replaced (normal phosphates), are alkaline to litmus. While all varieties of the phosphates of sodium, potassium, and ammonium are freely dissolved by water, of the alkaline earth metals only the superphosphates are at all freely soluble. The monohydrogen and normal phosphates of calcium, magnesium, and, we may add, of barium, are scarcely taken up by water.

If to a weak solution of, say, sodium - dihydrogen - phosphate (NaH_2PO_4) calcium chloride or barium chloride be added, no precipitation occurs; the corresponding salts of these latter metals being comparatively soluble. On the other hand, from a solution of di-sodium-monohydrogen-phosphate (Na_2HPO_4) nearly all of the phosphoric acid is precipitated on the addition of a calcium or barium salt, in the form of the corresponding monohydrogen phosphate of the alkaline earth. In any mixed solution of di- and mono-hydrogen phosphates, the amount of phosphoric acid which is left unprecipitated by, say, barium chloride, is a measure of the proportion of the dihydrogen phosphate originally present. Now, if we apply this test to urine of average acidity, we find that about 60 per cent. of the total phosphoric acid remains in solution after the addition of the barium chloride. We are justified in concluding, therefore, that acid dihydrogen phosphates are present in about this proportion; a fact in itself sufficient to account for the acid reaction of the fluid towards litmus. The composition of the barium precipitate from an acid urine proves that the remaining phosphoric acid is mainly in the form of monohydrogen salts.

If, now, we suppose the excretion to receive an increased quantity

of the acid products of metabolism—what will be the effect on the distribution of bases? It has been shown experimentally, that if to a mixed solution of mono- and di-hydrogen phosphates, a mineral acid (such as sulphuric acid) be added, in quantity not greater than is equivalent to the bases present in the monohydrogen form, no free acid is afterwards found in solution; but there will be an increase in the dihydrogen phosphates at the expense of the monohydrogen phosphates in proportion to the amount of acid added. Not only is this true of sulphuric acid; it has been shown that all the weaker acids or acid salts which are liable to reach the urine from the circulation (*e.g.* hippuric acid or acid oxalates) are able, when added to a solution of the mixed phosphates, to remove base from the monohydrogen form, and so to produce almost an equivalent increase in the acid phosphates. So long, therefore, as both these types of phosphate exist side by side (and they are always found together in acid urine), we can assume that the acidity of the fluid is due to the acid phosphate, and practically to that alone. The simultaneous existence of the monohydrogen form will be seen to be a guarantee of this, as it will have to disappear by interchange of bases, before any other urinary constituent can begin to exert its own proper acidity to any appreciable extent.

When the urine reacts alkaline to litmus, the alkalinity may under different circumstances be due (1) to excess of basic phosphates, (2) to carbonates of the fixed alkalies, or (3) to ammonium carbonate.

Determination of the acidity.[1]—It is, as we have seen, not difficult to assign the acidity of the urine to its proper cause; but when we endeavour to discover a method by which to estimate the *degree* of acidity, and especially a mode in which to express its value numerically, we meet with considerable difficulties.

In the case of a fluid the acidity of which is due to a strong acid, capable of forming stable salts with the alkalies, the ordinary methods of acidimetry yield a determinate result, and the estimation of acidity is one of the simplest operations in chemistry. We have but to note the amount of a standardised solution of alkali which is sufficient exactly to neutralise the acid present, and the point of neutralisation is given sharply and exactly by the colour change which occurs in the presence of one of many available indicators. In urine, owing to the unstable phosphate equilibrium, and the presence of other salts which influence the result, the process is much less determinate. To litmus, as already stated, a dihydrogen phosphate, *e.g.* NaH_2PO_4, is acid, while Na_2HPO_4 and Na_3PO_4 are alkaline; but no mixture of these salts can be found which is, strictly speaking, neutral to litmus paper.

If we start with a urine acid to litmus and gradually add alkali, we at last reach a point when the fluid shows a paradoxical behaviour. It makes red litmus paper tend to blue, and blue paper tend to red, inducing in fact a somewhat violet colour in both. It reacts at once acid and alkaline. This occurs when the monohydrogen phosphates, which during the addition of alkali are gradually increased at the expense of the dihydrogen salts, have come to bear a certain proportion to the latter.

Many urines exhibit this so-called amphoteric reaction without the

[1] I have discussed this subject at what may seem disproportionate length, but the problem involved illustrates well the complexity of chemical conditions in the urine; and much has been written upon it of late on what I venture to believe are erroneous lines.

addition of extraneous alkali. The reaction usually betokens that the monohydrogen salts exist in larger proportion than the dihydrogen, but it prevails through a considerable range of variations in this proportion; its exact limits depending in part upon the delicacy of the litmus paper used. Throughout the range of the amphoteric reaction a solution of litmus, actually mixed with the fluid, retains a violet or neutral colour practically unchanged.

Heintz attempted to explain this amphoteric reaction as follows. The red colouring matter of litmus acts as a dibasic acid, forming with bases, either unsaturated salts which are violet, or saturated salts which are blue. From the saturated salt the dihydrogen phosphates may extract half the base, leaving the unsaturated violet salt. The monohydrogen phosphates, on the other hand, may yield to the red acid substance sufficient base to form also the violet compound. In an amphoteric mixture the affinities are so balanced that this violet compound can alone exist. When, however, the acid phosphate is present in sufficient excess, it removes all the base and leaves the red free acid; with a large excess of the more basic phosphate, on the other hand, the litmus acid obtains its full complement of base and forms its blue salt.

With other indicators we can obtain a colour change at a more definite point during the process of alkalisation of an acid urine, and to the use of these we shall shortly return. But it should be made clear that only in the interaction between a "strong" acid and a "strong" base is the colour change, with an indicator, synchronous (or approximately synchronous) with the final replacement of all the acidic hydrogen atoms by the base. From this special case we have come to attach a definite value to the expression "degree of acidity," which is not found when we are dealing with such a substance as phosphoric acid. The "acidity" is here a quantity varying with the indicator used. The coloured indicator is itself an unstable compound which, in the play of acid and basic affinities, suffers a definite change when a certain point of equilibrium is reached. This point will depend upon the relative stability of the indicator and of the phosphates with which it is in contact, and may or may not occur simultaneously with the removal of all replaceable hydrogen from the latter.

The "degree of acidity" of a certain quantity of acid phosphate, in solution by itself, will be greater than that of an equal quantity mixed with a proportion of the more basic phosphates; and this is true, no matter what the indicator used. During the process of neutralisation by the standard alkali, the proportion of the more basic phosphates is gradually increased until the tendency of these to affect the indicator in one direction eventually balances the action of the acid phosphate in the opposite direction. This "neutral" point will evidently be reached the sooner, if some basic salt was originally present before titration was commenced.

Such considerations as these have led to a proposal to estimate the acidity of urine, not by simple titration, but by actually determining the proportion between the acid phosphates and the more basic phosphates present. For this purpose, Lieblein,[1] after a careful study of the matter, has recommended the process of Freund, which is an application of the barium precipitation method referred to above. The total phosphoric acid

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xx. S. 52–88. In this paper a criticism of other methods will be found.

is first determined in the original urine; that existing as monohydrogen phosphates is then removed by precipitation with barium chloride, and that present as acid phosphates is finally determined in the filtrate.[1]

But how exactly are we to express the urinary acidity in terms of the results so obtained?

Some recent writers have denoted the acidity by the figure expressing simply the ratio of acid phosphates to total phosphates.[2] If the P_2O_5 in the former be (say) 54 per cent. of the total P_2O_5, the relative acidity of the urine is to be called 54; if in another case it is only 27 per cent., the acidity is to be considered as half that in the first case.

Such a procedure seems to be wholly misleading. If of two specimens of urine one contains twice as much acid phosphate as the other, but at the same time twice the amount of the monohydrogen salt, the acidity, expressed in the above manner, will be the same in each case.

Such urines will certainly not *behave* as if of equal acidity, nor will they indicate the same acid production within the body.

We may here illustrate what we mean by the expression "behave as if of equal acidity." One of the most important results of a high grade of acidity is a tendency for the urine to deposit its uric acid in the free condition. In a later section, dealing with the urates (*q.v.*), the mechanism of this separation will be discussed. We shall find that one essential step in the process consists in the conversion of certain less acid urates (biurates) into more acid urates (quadriurates).

Now it is the acid phosphates which bring this change about, by removing base from the first form of urate, themselves becoming, of course, converted *pari passu* into more basic phosphates. But the latter, as they increase in quantity, tend to yield back the base to the quadriurates, so that a point is possible when the whole system will be in equilibrium. The less acid the urine, the sooner is this point reached. A little consideration will show that the "degree of acidity," from this point of view (and it is an important aspect), will be a function *both of the absolute amount of the acid phosphates, and of the ratio they bear to the total phosphates.* But we are hardly in a position to express the acidity *quantitatively* in terms of these two factors, because we do not know precisely at what stage the urates and phosphates are in equilibrium. It is probable, in fact, that the point of equilibrium is different for each of the diverse changes which may occur in the urine, as a result of its acidity, just as it is different for the colour change in diverse indicators. No more striking instance of the relativity of the phenomena involved could be given than a fact we shall discuss under the head of the pigments. Urinary hæmatoporphyrin is always found in the so-called alkaline form; and if we add to any normal urine either neutral or acid hæmatoporphyrin, we find that it immediately assumes the alkaline form. Equilibrium in this case is only attained when base has been transferred to the pigment from the *acid* phosphate. If, then, hæmatoporphyrin had happened to be our only available "indicator," we should have said that urine was normally an alkaline fluid!

The whole source of the difficulty we have been discussing is found in the fact that the terms "degree of acidity" or "degree of alkalinity" are unscientific, though convenient, modes of expression. With increase of knowledge, they will be replaced by expressions denoting the actual

[1] For the principles of this determination, see p. 633. An error of some 3 per cent. has to be allowed for, due to a conversion of monohydrogen into dihydrogen phosphate in the process of precipitation.

[2] Cf. Hausmann, *Ztschr. f. klin. Med.*, Berlin, 1896, Bd. xxx. S. 350.

chemical energy of the system of mixed salts. The degree of acidity of the urine (or any analogous fluid) is in fact not an absolute quantity, but is wholly relative to the means which we employ to measure it. But by always employing the same means, be it noted, we may obtain relative results which are strictly comparable, and as an outcome of this somewhat difficult discussion, it may be suggested that we shall do well in the present state of our knowledge to continue to employ a simple titration method, by which we obtain comparable, if only relative, measurements. But we must employ an indicator which gives a more definite point of colour change than does litmus, and we must retain the same indicator for any one series of experiments; moreover, the nature of the indicator used must always be stated in stating the results. Phenolphthalein, and perhaps cochineal, will serve our purpose. If acid urine be gradually neutralised in the presence of the former of these, which is colourless when acid, a pink tinge is developed at a certain stage in the process, and we are justified in speaking of a specimen of urine which requires more alkali to produce this change as "more acid" than one which requires less.

What has been said in this section will have left a wrong impression if it be thought that such measurements are of no value. My endeavour has been to show that we have at present no means of expressing the acidity of the urine as an absolute quantity independent of the particular means adopted for measuring it. But, having chosen a method of estimation, and being careful always to use the same method, we may accurately follow the variations of urinary acidity, and obtain results with important bearings.

Variations in acidity.—The degree of acidity as determined by titration is, as we have seen, in the main, a resultant of two opposing factors; on the one hand, acid production in metabolism; on the other, the ingestion of unsaturated or unstable basic compounds, supplemented by the production of ammonia within the body. To these, however, a third factor must be added—the elimination of acids or bases respectively by other than renal channels.

The separation of the acid gastric juice and the consequent liberation of bases in the blood is associated with increased excretion of the latter in the urine. On the other hand, the flow of alkaline secretions —bile, pancreatic juice, etc.—diminishes the urinary bases.

From these considerations, the reasons for the variations in acidity commonly met with become clear. The acidity increases with increased proteid metabolism, with exercise, and with the consumption of food, when this contains a small proportion of bases—in particular, with flesh food. It diminishes when the food taken contains abundant bases. The compounds of organic acids with the alkaline metals, which are so plentiful in vegetable food, become oxidised in the body to carbonates, and the excretion of bases thence derived tends to alkalise the urine. From this follows the familiar fact that the urine of herbivorous animals is alkaline, and that human urine may become alkaline (though seldom continuously so) when a vegetarian diet is maintained.

The effect of the secretion of gastric juice is to produce what is called the *alkaline tide*. During the period of full gastric digestion the urine may become less acid, and may even (though this is rare) become alkaline to litmus. The occurrence of this phenomenon was first noted by Bence Jones.

It must not be supposed, however, that the post-prandial alkaline tide is a universal phenomenon. It will be easily seen that the effect of digestion upon the bases and acids of the blood must be somewhat complex. The flow of alkaline saliva precedes, and that of bile and pancreatic juice rapidly follows, the gastric secretion; and these, by removing bases, tend to neutralise the effect of the removal of acid *viâ* the stomach. From this cause, and from the increased proteid metabolism induced by the food, it not infrequently happens that the urinary acidity is from the first raised, instead of lowered, after a meal.

According to Quincke, a periodic variation of acidity may occur during the day, independently of food ingestion,[1] and in my experience this is a more constant phenomenon.

Gruber found that the urine may become alkaline after a large consumption of sodium chloride, and Rüdel[2] has recently stated that the pure diuresis produced by such substances is in itself capable of inducing this result. This may be true, under the somewhat extreme conditions of experiment, but when the urinary constants are followed under natural conditions from hour to hour, it is not found that the quantity of urine passed during a given period has any regular influence on the *total acidity* of the same period.[3]

Pathologically, a tendency to alkalinity is said to be found in most conditions of debility, and especially in some types of anæmia; probably from diminished secretion of gastric juice, and from diminished general metabolism. A process quite distinct from this occurs when, under the influence of organisms (especially the *Micrococcus ureæ*), the urea and uric acid of the urine are hydrolised into ammonium carbonate. In cystitis this may occur in the bladder, and the urine is voided alkaline with ammonia.

The acidity is especially high in scorbutic urine, and is increased to a greater or less degree in some forms of dyspepsia, in diabetes, leukæmia, and in pernicious anæmia.

The Nitrogenous Compounds.

(a) Total nitrogen.—The urinary nitrogen amounts, on an average, to 15 grms. in the twenty-four hours. This comprises by far the greater part of the nitrogenous loss to the body; less than 1 grm. being eliminated through the intestinal secretions and all other channels combined.

Pathologically, the amount may be greatly increased; 20 to 25 grms. per diem is frequently observed in fever, and in severe forms of diabetes 50 grms. and upwards may be daily eliminated. On the other hand, a marked diminution of the amount is seen in the condition of contracted or granular kidney.

Under normal conditions, the urinary nitrogen is distributed in various compounds in the following proportions: About 86 per cent. of the whole is found in the form of urea; about 3 per cent. as ammonia, 3 per cent. as creatinin, 2 per cent. as uric acid and the allied xanthin bases; while the remaining 6 per cent. is present, in varying proportions, in hippuric acid, in indol and skatol, in the urinary nucleo-albumin, in the pigments, and in minute quantities of other constitutents.

The total nitrogen is *estimated* by one of the many modifications of Kjeldahl's process, which is founded on the fact that organic substances,

[1] *Ztschr. f. klin. Med.*, Berlin, 1884, Bd. vii. Suppl. 22.
[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1892, Bd. xxx. S. 41.
[3] Cf. Hausmann, *Ztschr. f. klin. Med.*, Berlin, 1896, Bd. xxx. S. 362.

when heated with concentrated sulphuric acid, become oxidised, and all the nitrogen (except such as may be originally present in combination with oxygen) is converted into ammonia. The resulting ammonia is liberated by the addition of caustic alkali, and distilled into a measured quantity of standard acid; its amount being finally determined by titration. Kjeldahl's method gives admirable results with urine, and may be applied to 5 c.c. of the fluid.

(b) **Urea—$CO(NH_2)_2$.**—The presence of urea in the urine was first demonstrated by Rouelle in 1773. It is the chief end-product of nitrogenous metabolism in all mammals, in amphibia, and in fishes. In 1828 it was obtained artifically by Wöhler, by heating the isomeric substance ammonium cyanate ($NH_4.CNO$).

The chemical constitution of urea is that of an amide of carbonic acid (carbamide).

Properties.—Urea crystallises in colourless needles or rhombic prisms, containing no water of crystallisation, and melting at about 130° C. It is freely soluble in alcohol, and still more so in water; in pure ether or chloroform it is insoluble. Urea, like other amides of acids, is neutral to litmus; but, owing to the presence of two ammonia residues in its molecule, it exhibits weak basic properties, and forms loose molecular compounds, analogous to salts, two of which are of practical importance.

Urea nitrate = $CO(NH_2)_2.NO_2.OH$.—This compound crystallises out when excess of pure nitric acid is added to a not too weak solution of urea; excess of the acid assists its separation, as it is less soluble in nitric acid than in water; crystallisation is accelerated by shaking and cooling the mixture. The fundamental form of the crystals is a rhombic table, of which the more acute angles measure 82°; but, by truncation of the angles, six-sided tablets are commonly formed, and these are apt to adhere together and overlap like tiles on a roof (Fig. 49). When rapidly heated, the crystals deflagrate. At 140° they decompose into nitrous oxide, carbon dioxide, and ammonium nitrate.

Urea oxalate = $CO(NH_2)_2.(COOH)_2$ — is formed in an analogous manner by mixing solutions of urea and oxalic acid; like the nitrate, this salt is less soluble in excess of the acid. Its crystals belong fundamentally to the same type as those of the preceding compound, but are apt to appear as thick short rhombic prisms (Fig. 49).

A crystalline combination of urea with phosphoric acid is also known, and others with various organic and inorganic acids.

Crystalline compounds are also formed with certain neutral salts; that with sodium chloride is said occasionally to form when urine is concentrated on the water bath. The compound with palladium chloride is very insoluble. A molecular combination with *basic mercury nitrate* is quite insoluble in water, and is of historic interest, as its formation is the basis of the classical method of urea estimation suggested by Liebig in 1853 (*vide infra*).

When fused and gently heated after fusion, urea yields *biuret* and *cyanuric acid*. Two reactions occur as follows:—

$$2NH_2.CO.NH_2 = \underset{\text{(biuret)}}{NH_2.CO.NH.CO.NH_2} + H_3N\text{; and}$$

$$3NH_2.CO.NH_2 = \underset{\text{(cyanuric acid)}}{C_3N_3(OH)_3} + 3(H_3N)$$

Its relations to ammonium carbonate and carbamate are very important from a physiological standpoint.

$$CO\begin{matrix}NH_2\\NH_2\end{matrix} + H_2O = CO\begin{matrix}ONH_4\\NH_2\end{matrix} + H_2O = CO\begin{matrix}ONH_4\\ONH_4\end{matrix}$$

(urea) (ammonium carbamate) (ammonium carbonate)

The two molecules of water necessary to form carbonate of ammonia are very readily taken up. Even at a temperature of 60° C., an aqueous solution of urea slowly develops ammonia (Leube[1]); while a boiling solution decomposes with considerable rapidity. Heated with water

Fig. 49.—Upper half, urea nitrate crystals. Lower half, urea oxalate crystals.

under pressure at 180°, the conversion into ammonium carbonate is quickly complete. A solution of pure urea may be evaporated at temperatures from 60° to 75°, without serious loss, but in the urine it is less stable. Quite appreciable proportions of its nitrogen are lost as ammonia when urine is evaporated, even at low temperatures. In the presence of free acids and bases, the hydrolysis occurs with still greater readiness, the ammonium carbonate formed being further decomposed by the reagent. Thus, on boiling urea solutions with acids, carbonic acid is given off; on boiling them with alkalies, free ammonia is evolved.

The hydrolysis is also induced by micro-organisms, as in the ammoniacal fermentation of urine. The *Micrococcus ureæ* is the best known of these; but other organisms are found in decomposing urine

[1] *Virchow's Archiv*, 1885, Bd. c. S. 552.

which can produce the same result. On the other hand, urine may develop many organisms which have no such power.[1] So long as the bacteria which induce the change are alive, the enzyme is closely associated with the living cell, and a filtered urine is ferment free (Sheridan Lea). But when the cells are dead, a ferment may be extracted from them which hydrolyses pure urea solutions.

While urea is thus easily converted into ammonium carbonate, the intermediate substance ammonium carbamate (formed by the action of dry CO_2 upon NH_3), if heated to 135°, or treated with alternating electric currents, splits up into urea and water. The hepatic cells have the power of dehydrolising ammonium carbonate itself to form urea. Nitrous acid and the hypobromites oxidise urea according to the following equations:—

(1) $CO(NH_2)_2+2NOOH=CO_2+2N_2+3H_2O$
(2) $CO(NH_2)_2+3BrONa=3NaBr+CO_2+N_2+2H_2O$

Separation of urea.—To prepare pure urea from urine, advantage may be taken of the insolubility of the nitrate. The urine is concentrated to a small bulk, and pure nitric acid is added in excess; the mixture being kept thoroughly cool during the addition of acid. The crystals are strained off by pouring through muslin, and freed from excess of acid by pressing between thick filtering paper. They are mixed with excess of barium carbonate, sufficient alcohol is added to form a paste, and the mixture dried on the water bath. On extracting the dried residue with absolute alcohol, a fairly pure solution of urea is obtained, from which crystals separate on evaporation.

I find that fine crystals may be prepared by the following simpler method. Half a litre of urine is evaporated to a thick syrupy consistence, and the residue is exhausted with hot absolute alcohol. The spirit is filtered and taken to dryness; and the residue extracted on the water bath with successive quantities of pure acetone, which should be filtered while hot. The mixed acetone extracts are evaporated nearly, but not quite, to dryness. On cooling, fine white crystals of urea separate, any pigment present remaining in solution in the small quantity of the solvent which is allowed to remain. The crystals may be washed with cold acetone.

Tests.—For the detection of urea, the formation of the characteristic crystals of the nitrate or oxalate (and especially of the former) is of practical value. On the small scale the process of crystallisation may be watched under the microscope; a drop of the suspected fluid, after concentration if necessary, and another of nitric acid, being allowed to run together on a glass slide.

The formation of biuret is an excellent test for urea, if the crystals are first obtained in moderate quantity and fairly pure. After heating them, as described above, the residue is dissolved in water, excess of caustic alkali is added, and one or two drops of a dilute copper sulphate solution. A pink colour is produced like that given by peptones under like circumstances ("biuret reaction").

Estimation of urea.—No method is known by which urea can be separated, as such, from the urine in a quantitative manner. The ease with which it is hydrolised is a fundamental difficulty in the way of such quantitative isolation. We can, however, find precipitants for the other nitrogenous constituents, and a determination of the remaining nitrogen after the removal of these gives the best available measure of the urea.

[1] For information on this subject, *vide* Leube and Graser, *Virchow's Archiv, loc. cit.*; and Warrington, *Journ. Chem. Soc.*, London, 1888, vol. i. p. 727.

The most satisfactory of the methods based upon this principle is that of Mörner and Sjöquist.[1] To carry out this process, 5 c.c. of the urine is treated with an equal volume of a saturated solution of barium chloride containing 5 per cent. of caustic baryta; 100 c.c. of an alcohol-ether mixture (2–1) is added, and the whole allowed to stand for twenty-four hours in a closed flask. After filtering from the precipitate the solution is evaporated at low temperatures (below 60°), and a determination of nitrogen made, by Kjeldahl's method, in the residue. By the precipitation thus described all nitrogenous substances are removed except urea and ammonia, while the last is got rid of during the evaporation of the filtrate. The percentage of nitrogen found multiplied by 2·143 will give the percentage of urea.

When less accuracy is required, the well-known process of Knop[2] and Hüfner is now universally employed. This depends on the decomposition of urea by the action of hypobromites; the nitrogen which is evolved being measured in a graduated tube, and the urea calculated from the amount thus found. The equation for this reaction is given above (p. 583). The solution of sodium hypobromite employed contains excess of caustic alkali, so that the carbon dioxide which is formed simultaneously with the free nitrogen, is retained in solution as carbonate of sodium. Only some 92 or 93 per cent. of the total nitrogen present as urea is obtained in this process, the remainder being converted into cyanates. On the other hand, the uric acid, creatinin, and other nitrogenous substances present yield a proportion of their nitrogen, so that part of this error is counterbalanced. Many varying influences affect the result, however; diabetic urine, for instance, is said to yield a greater proportion of its total nitrogen, owing to the effect of the sugar present. It should, in fact, be clearly understood that the hypobromite process, while of great convenience and of sufficient accuracy for clinical and many other purposes, does not give a scientific measure of the urea. The calculation of its results is best made by taking each 37·1 c.c. of nitrogen measured at ordinary temperatures as equivalent to one decigramme of urea.[3]

The titration method of Liebig referred to on p. 581 is now of little more than historical importance, though it was used in all the older work upon metabolism. It depended in principle on the fact that urea, under carefully defined conditions, forms a definite insoluble compound with basic mercuric nitrate. A standard solution of nitrate of mercury was added to the urine until the whole of the urea was precipitated in this form, the end-point being marked when a drop of the urine gave a yellow colour with sodium carbonate (indicating excess of mercury). The modifications necessary for accuracy have been carefully worked out by Pflüger and others; in its perfected form, however, the process becomes one for the estimation of the total nitrogen of the urine rather than for the urea only, and for this purpose it is entirely superseded by Kjeldahl's method (*supra*, p. 580).

The *variations* in the *quantity* of urea present in the urine are dealt with in the article on metabolism, where their cause is discussed. The average quantity excreted by a healthy adult man under normal circumstances is about 30 grms. per diem; that is to say, the urine will contain about 2 per cent. Its absolute amount is necessarily increased by all causes which stimulate nitrogenous metabolism, but the *proportion* which the urea bears to the other nitrogenous constituents is an independent variable (*vide infra*).

[1] *Skandin. Arch. f. Physiol.*, Leipzig, 1891, Bd. ii. S. 438; *Jahresb. u. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xxi. S. 168; cf. also Bödtker, *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xvii. S. 146.

[2] The original description by Knop will be found in *Chem. Centr.-Bl.*, Leipzig, 1860, S. 244. Details of various modern modifications are found in most practical handbooks.

[3] Cf. A. H. Allen, "Chemistry of Urine," p. 148.

According to Tschlenoff,[1] if the urea excretion after a meal rich in proteids be estimated from hour to hour, it will be found to exhibit two maxima. The first occurs at the third or fourth hours, and the second at the sixth or seventh. These he considers to indicate the absorption of peptones from the stomach and intestine respectively. If peptones be given instead of ordinary proteids, the maximum is reached by the second hour. Marès,[2] on the other hand, found that after an isolated meal the maximum of urea excretion was not reached till the ninth hour. Kobler [3] has found that simple diuresis under normal circumstances is not accompanied by increased excretion of urea.

(c) **Ammonia.** — The urine of man and of carnivorous animals invariably contains small quantities of ammonium salts. They may be absent, however, from that of herbivora. The quantity in human urine is about 0·7 grm. NH_3 per diem; the variations in health extending from about 0·3 to 1·2 grms.[4]

The ingestion of ammonium carbonate, or of organic ammonium compounds susceptible of oxidation in the body, does not increase the excretion of ammonia, for the nitrogen of such compounds is excreted wholly as urea. If, however, stable salts of ammonium, such as the chloride, are given, they appear (in the case of carnivora, at any rate) as such in the urine.

Apart from such direct ingestion of stable ammonium salts, the excretion of ammonia depends almost entirely upon that question of adjustment between acid production in metabolism and the supply of bases in the food which was discussed in the section devoted to the acidity of the urine (*q.v.*). Ammonia formation is the physiological remedy for deficiency of bases.

When acid production is excessive (a condition especially seen in certain forms of diabetes), or when mineral acids are given by the mouth, the urinary ammonia increases at the expense of the urea. When the bases are in excess, whether from the nature of the food or from the administration of alkalies, the ammonia disappears, and a corresponding amount of urea is excreted in its place. From this it follows that little or no ammonia is found in the urine of herbivora; and that, in man, flesh food raises the quantity, and vegetable food diminishes it.[5]

From the abundance of bases in their food, it is very difficult, by any means, to increase the urinary ammonia of herbivora. If, for example, abundant ammonium chloride be given to a rabbit, together with a normal supply of vegetable food, its urinary ammonia is but little increased.[6] By double decomposition with sodium carbonate in the tissues, ammonium carbonate and sodium chloride are formed, and the former is excreted as urea.

It would seem that the organisation of the herbivora does not permit of a supply of ammonia to neutralise acids when given in excess. Thus, most herbivorous animals are said to be much more susceptible to poisoning by acids than are the carnivora.

[1] Abstract in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1896; cf. also Veragutt, *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 112.

[2] *Jahresb. ü. d. Leistung. . . . d. ges. Med.*, Berlin, 1887, Bd. i. S. 145.

[3] *Wien. klin. Wchnschr.*, 1891, Nos. 19, 20.

[4] Neubauer, *Journ. f. prakt. Chem.*, Leipzig, 1852, Bd. lxiv. S. 177. These figures are confirmed by numerous later observers.

[5] Salkowski and Munk, *Virchow's Archiv*, 1877, Bd. lxxi. S. 500; also Gumlich, *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xvi. S. 19.

[6] E. Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 26.

To demonstrate the presence of the small quantities of ammonia in human urine is not easy, owing to the ready production of the base by hydrolysis of urea, which must, obviously, lead to error. We must employ a method analogous to that used for its estimation.

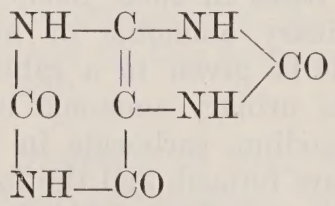
Estimation of ammonia (*Schlösing's method*).—Twenty-five c.c. of urine are placed in a basin with vertical sides, and about 20 c.c. of milk of lime are added. A glass triangle is placed over the basin, and, upon it, another small vessel containing 20 c.c. of one-fifth normal sulphuric acid. These stand upon a glass slab, and are covered with a bell-shaped glass cover, fitting air-tight on the slab. The ammonia is liberated by the lime, without any decomposition of other nitrogenous constituents, and, in the course of three days, the whole is absorbed by the sulphuric acid, the degree of neutralisation being afterwards estimated by titration. If dilute hydrochloric acid be used instead of sulphuric, it may, after the experiment, be evaporated to dryness on the water bath, and the residue taken up with a small quantity of water. Platinic chloride added to this solution will demonstrate the presence of ammonia, by giving a yellow crystalline precipitate of ammonio-platinic chloride.

Pathologically, the urinary ammonia may be increased, not only after the manner we have discussed, by abnormal acid production (as in diabetes and fevers), but also by conditions which reduce the proper activity of the hepatic cells, whereby the dehydrolysis of ammonium carbonate into urea is less complete than normally.

(d) **Uric acid.**—Uric acid was first separated from human urine by Scheele, in 1776. It is present in the urine of most mammals, though from that of the dog and cat it has been shown to be frequently absent. In man the daily output in the urine varies considerably (from 0·2 grm. to 1·4 grm.), the average amount being 0·8 grm.

Chemical constitution.—Rightly to appreciate the physiology no less than the chemistry of uric acid, its close relationship to urea should be clearly understood. It yields the latter easily by a combined process of oxidation and hydrolysis. It belongs, in fact, to the class of substances known as *diurëides*, in which the residue of two urea molecules are united to a carbon-containing nucleus. In the case of uric acid this nucleus contains a chain of three carbon atoms.

The constitutional formula first suggested by Medicus—



has now received ample confirmation from the synthetic production of the acid by Horbaczewski,[1] and by Behrend and Roosen.[2]

The ureides are, in general, produced by the condensation of hydroxy-acids with urea. The hypothetical acid, which would yield uric acid by such simple condensation, would be a trihydroxyacrylic acid; but this has never been prepared.

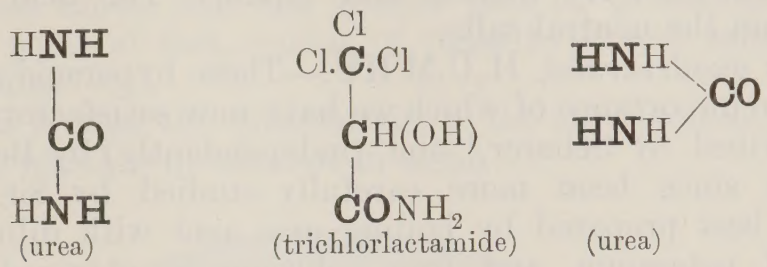
Lactic acid also contains a three-carbon chain in its molecule, and, because of the important physiological relationships of this acid, it is of special interest to find that uric acid can be synthesised by linking urea

[1] *Monatsh. f. Chem.*, Wien, 1887, Bd. viii. S. 201, 584.
[2] *Ber. d. deutsch. chem. Gesellsch.*, 1888, Bd. xxi. S. 999.

residues on to a nucleus derived from lactic acid. This Horbaczewski succeeded in doing by heating urea with trichorlactamide :—

$$CCl_3CH.OH.CO.NH_2+2(NH_2)_2CO=C_5H_4N_4O_3+NH_4Cl+2HCl+H_2O$$

The simple changes involved in this reaction will be more clearly seen on examination of the following graphic scheme :—



The groups printed in thick type unite to form uric acid ; the atoms represented in thinner type split off to form respectively a molecule of ammonium chloride, two molecules of hydrochloric acid, and one of water.

Uric acid is formed also when glycine is heated with urea (Horbaczewski), but the molecular changes involved are not so simple as those shown above, and the yield is not so good. In Behrend and Roosen's synthesis the nucleus is primarily derived from acet-acetic ether, and the urea residues are linked on separately at two different stages in the synthetic process.

Properties.—Pure uric acid forms a white powder, which is made up of small rhombic crystals, of more or less prismatic or tabular type. Its crystalline forms become very diverse in the presence of impurities, and when it separates from the urine, the crystals, which are then always coloured, take shapes which depend to a large extent upon the nature of the pigment associated with them[1] (Figs. 50 and 51).

In cold water it is very insoluble, only dissolving to the extent of about 1 part in 15,000. A litre of boiling water takes up about half a gramme. Ether and alcohol do not dissolve it. It dissolves in oil of vitriol without decomposition, and from the solution a crystalline sulphate separates on freezing the mixture. By this process pure uric acid may be obtained from contaminated specimens, the sulphate being resolved into its constituents when treated with water.

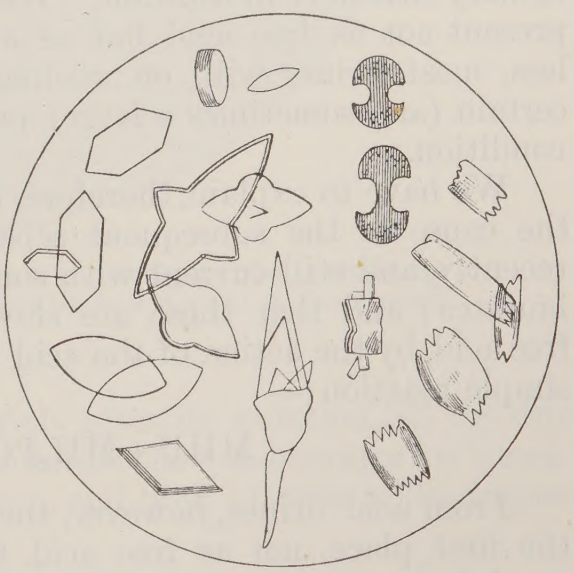


Fig. 50.—Uric acid.

It acts as a somewhat weak dibasic acid, but forms three orders of salts.

1. The *neutral urates*, $M'_2\overline{U}$, have an intense caustic taste and are very unstable. They are decomposed by carbonates and even by the carbonic acid of the air. As they are only produced in the presence of caustic alkalies, and cannot exist in the presence of carbonates, it is un-

[1] A. E. Garrod.

likely that they can, under any circumstances, occur as physiological products.[1]

2. The acid *urates* or *biurates*, $M'H\bar{U}$, are the most stable of the compounds of uric acid. They are prepared by dissolving the acid at boiling heat in weak solutions of the alkaline carbonates, from which they separate, after cooling, in stellar crystals. These and the foregoing salts were first studied by Bensch and Allan.[2] The acid urates are less soluble than the neutral salts.

3. *The quadriurates*, $H_2\bar{U},M'H\bar{U}$.—These hyperacid salts, for the existence and importance of which we have now satisfactory evidence, were first described by Scherer,[3] and (independently) by Bence Jones,[4] but they have since been more carefully studied by Sir Wm. Roberts.[5] They are best prepared by boiling uric acid with dilute solutions of acetate of potassium, and from solutions so obtained a quadriurate separates, as an amorphous precipitate, or in crystalline spheres.[6] They are very unstable, and when treated with water they split up into biurates and free uric acid. Owing to this instability it is impossible to determine directly their solubility in water; but they are probably less soluble than the preceding order of salts, as a strong solution of a biurate, when treated with acid-sodium phosphate, gives an abundant precipitate of a quadriurate. From analogy we might expect the three orders of salts, as described, to be in a descending series as regards solubility.

Condition of uric acid in the urine: its spontaneous separation.—Coloured indicators which are sensitive to free uric acid give no indication of its presence, as such, in freshly-passed urine. Again, the quantity of uric acid present is generally greatly in excess of what would dissolve in a volume of water equal to that of the urine. The presence of neutral salts, and also, according to Rüdel,[7] of urea, enhances this solubility, but not to a degree necessary for the retention of all the urinary uric acid in solution. We are led to expect, therefore, that it is present not as free acid but as a more soluble compound. Nevertheless, most urines will, on cooling and prolonged standing, deposit a certain (and sometimes a large) proportion of their uric acid in a free condition.

We have to explain, therefore, the nature of the original solution and the cause of the subsequent separation. The view generally held till recently, and still current with some authorities, is that the acid exists as biurates; and that these are slowly decomposed, with liberation of the free acid, by the action of the acid phosphates, according to the following simple reaction:—

$$M\bar{H}\bar{U} + MH_2PO_4 = H_2\bar{U} + M_2HPO_4$$

From acid urines, however, the uric acid is frequently deposited, in the first place, not as free acid, but in the form of urates, forming a precipitate which has long been known as the "lateritious deposit."

A careful study of the chemistry of this deposit has led Sir William Roberts to conclude that the above equation does not rightly, or at least

[1] Roberts.
[2] *Ann. d. Chem.*, Leipzig, 1848, Bd. lxv. S. 181.
[3] Neubauer ü. Vogel, "Analyse des Harns," 9th edition, S. 192.
[4] *Journ. Chem. Soc.*, London, 1862, vol. xv. p. 8.
[5] "Croonian Lectures," 1892.
[6] *Ibid.*
[7] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1892, Bd. xxx. S. 469.

does not completely express the chemical mechanism of uric-acid solution and precipitation.[1]

The urate deposit is amorphous, but on treatment with water it is found to decompose, part of its uric acid being set free in crystalline form and part going into solution (Fig. 51). But this is a property which was stated above to be specially characteristic of the quadriurates, and closer examination shows that the greater part of an amorphous urate deposit does, in point of fact, consist of those hyperacid salts, and not of ordinary biurates.

Roberts' view is that the quadriurate is the only physiological type of uric acid salt, whether in blood or in urine.

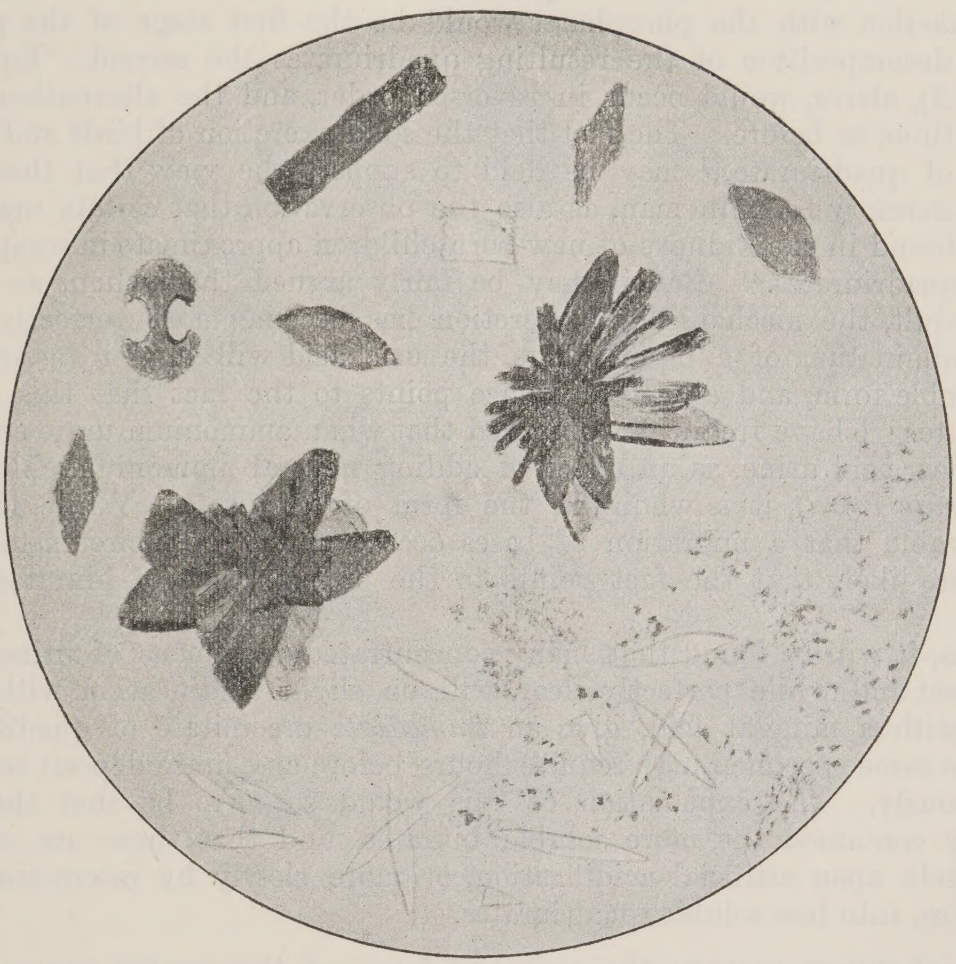


FIG. 51.—Uric acid.—In the lower half of the figure the crystals are shown as they separate when a quadriurate deposit is decomposed with water.

In the normal acid urine, immediately after its secretion, all the uric acid is in this form. But in aqueous solution the quadriurates are necessarily in a state of unstable equilibrium, and tend at once to decompose according to the equation—

$$(1)\ \mathrm{MH\bar{U},H_2\bar{U}} = \mathrm{MH\bar{U}} + \mathrm{H_2\bar{U}};$$

half the uric acid being precipitated and the other half remaining in solution as biurates. But the latter are in the presence of acid phosphates, and this fact again involves a condition of unstable equilibrium; the following change occurring—

$$(2)\ 2\mathrm{MH\bar{U}} + \mathrm{MH_2PO_4} = \mathrm{MH\bar{U},H_2\bar{U}} + \mathrm{M_2HPO_4}.$$

[1] Roberts, *loc. cit.*

In fact, quadriurates are thus re-formed, and become subject to the same influences as before. "These alternating reactions—breaking up of quadriurates by water into biurates and free uric acid, and recomposition of quadriurates by double decomposition of biurates with monometallic phosphate—go on progressively, until all the uric acid may be set free."

The quadriurates, therefore, are of great importance in the chemistry of urinary uric acid, and beyond all doubt form an intermediate step in the liberation of the free acid itself. The evidence that they are the form in which the acid is actually excreted seems to be less conclusive. It is clear that the alternating reactions just discussed would go on, whether the salts which leave the renal tubules are quadriurates or biurates. In the latter case, the interaction with the phosphates would be the first stage of the process, and the decomposition of the resulting quadriurates the second. Equations (1) and (2), above, would occur in reversed order, and the alternation would then continue as before. The fact that the solid excretion of birds and snakes consists of quadriurates,[1] may be held to support the view that these salts are the excretory form in man, as also the observation that certain urate concretions found in the kidneys of new-born children approximate in composition to the quadriurates.[2] But it may be fairly argued that when, as in the human adult, the mechanism of excretion has become more perfectly suited to the elimination of a liquid urine, the uric acid will tend to assume the more soluble form, and all the evidence points to the fact that this form is the biurate. I have frequently observed that when ammonium urate separates from a clear acid urine, as an effect of adding neutral ammonium chloride in excess (*vide infra*), it is wholly in the form of a biurate. While it is not inconceivable that a migration of bases occurs under these circumstances, it is far more likely that the fact points to the pre-existence of biurates in the urine.

Again, it will be found that many concentrated specimens of urine, when first passed and while perfectly clear, will, on slight acidification with acetic acid or with a mineral acid, give an *immediate* precipitate of quadriurates, while the same specimen may require hours before any urate deposit separates spontaneously. The explanation of this would seem to be that the urine originally contained the more soluble biurates, and that these are changed immediately upon artificial acidification, or more slowly by interaction with phosphates, into less soluble quadriurates.

But whatever may be the primary form of the urates present, it is in any case important to recall the facts discussed on p. 578. The reaction between urates and phosphates is a reversible one; with acid-phosphates, biurates yield quadriurates; with basic (monohydrogen) phosphates, quadriurates yield biurates. With a certain proportionate mixture of the two types of phosphate the uric acid salts will be therefore in equilibrium.

In many urines this equilibrium between the phosphates and urates is established, and the determining reactions described above, therefore, cease before all the uric acid is liberated. In others, where the proportion of monohydrogen phosphate is at the outside large, the equilibrium occurs early, and little or no free uric acid separates. Only when the original excess of acid phosphate over basic phosphate reaches an adequate value is the whole of the uric acid set free. In other

[1] Roberts, *loc. cit.*

[2] Flensburg, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1894, Bd. xxiii. S. 581.

words, the chief factor which determines the precipitation of uric acid is the degree of acidity of the urine. Roberts has found that two other agencies exert an influence over this precipitation—the pigmentation of the urine, and its comparative richness or poverty in salines. Other things being equal, a specimen which is poor in pigments on the one hand, or in neutral salts on the other, will exhibit a special tendency to deposit its uric acid in crystals. But while the question of acidity affects that stage of the process which consists in the change from biurates to quadriurates, the pigmentation and percentage of salts affect rather the change from quadriurate to free acid. The urinary pigments and the neutral salts inhibit the decomposition of quadriurates by water.

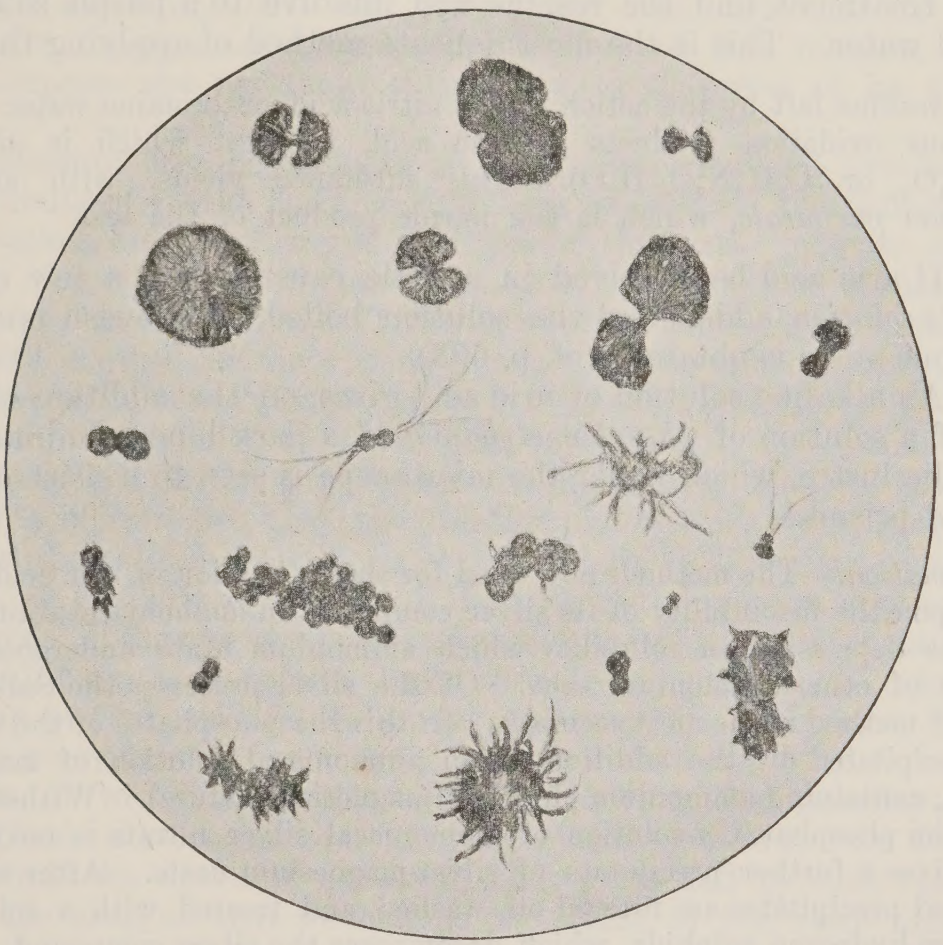


FIG. 52.—Upper half, ammonium urate. Lower half, sodium urate.

Upon standing, some specimens of urine deposit urates, not as amorphous quadriurates, but as crystalline biurates. Ammonium urate is frequently to be seen in the deposit from alkaline urine in the form of roughly dumb-bell-shaped masses; and in concentrated specimens sodium urate forms the so-called thorn-apple crystals (Fig. 52).

Isolation of uric acid from the urine.—If the urine be acidified with hydrochloric acid, much of its uric acid separates in pigmented crystals, which tend to adhere to the sides of the vessel. These can be easily identified by the microscope. But for the purpose of applying the characteristic tests, a supply of uric acid may be more conveniently and quickly obtained by adding crystals of ammonium chloride to the urine till near saturation, and then a few drops of strong ammonia. The precipitate which falls is at once filtered off, washed from the filter with a little hot water, and warmed with a few drops of hydrochloric

acid. After cooling, the crystals of uric acid which fall may be washed by decantation.

Tests and reactions—(*a*) *The murexide test.*—If a small quantity of uric acid be placed upon a watch glass, a little strong nitric acid, or a few drops of bromine water added, and the whole taken to dryness upon the water-bath, an orange-red residue is obtained which, if touched with a drop of ammonia, yields a fine purple colour. If a minute quantity of sodic-hydrate solution be subsequently added, the purple colour changes to blue; while, on warming the alkaline solution, all colour is discharged. The water-bath should always be used for evaporation in applying this test, and if the watch glass be allowed to remain on the bath for a considerable time, after evaporation is complete, a red colour will develop without further treatment, and the residue will dissolve to a purple solution in distilled water. This is the most delicate method of applying the test.

The residue left by the action of the nitric acid or bromine water consists of various oxidation products of uric acid, amongst which is *alloxantin* ($C_5H_6N_4O_8$ or $C_8H_4N_4O_6.H_2O$). This substance yields, with ammonia, *ammonium purpurate*, which is the purple product of the test.

(*b*) If uric acid be dissolved in a little caustic soda, a few drops of *Fehling's solution* added, and the solution boiled, a yellowish precipitate of cuprous oxide is obtained (cf. p. 608).

(*c*) An alkaline solution of uric acid gives, on the addition of a few drops of a solution of *phosphomolybdic acid*, a dark blue precipitate with a metallic lustre, which under the microscope is seen to consist of small six-sided prisms.[1]

Estimation.—The methods now used for the estimation of uric acid depend either upon the insolubility of its silver compound in ammoniacal solutions, or upon the depression in solubility which ammonium urate undergoes in the presence of other ammonium salts. Of the silver processes the Salkowski-[2] Ludwig[3] method is the most accurate. In this the phosphates of the urine are first precipitated by the addition of an ammoniacal solution of magnesium chloride, containing ammonium chloride (magnesia mixture). Without filtering off the phosphates, a solution of ammoniacal silver nitrate is next added, which gives a further precipitate of silver-magnesium urate. After standing, the mixed precipitates are filtered off, washed, and treated with a solution of potassium-hydrogen sulphide, which decomposes the silver compound, forming silver sulphide and potassium urate. The black precipitate of the former is filtered off, and the uric acid liberated in the filtrate by the addition of hydrochloric acid. It is finally separated by filtration and weighed.

The writer[4] has modified the previous methods employed for the separation of uric acid as ammonium urate in such a way that the precipitation is absolutely complete, and the results are as accurate as those of the foregoing method, while much more easy to obtain.[5] The urine (100 c.c.) is saturated with chloride of ammonium, and allowed to stand for two hours, when the resulting ammonium urate precipitate is filtered off, washed from the filter with hot water, and the uric acid liberated by warming with hydrochloric acid. After standing it is filtered off, washed, and weighed.

[1] Offer, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1894, Bd. viii. S. 801.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 210.
[3] *Ztschr. f. anal. Chem.*, Wiesbaden, 1885, Bd. xxiv. S. 637.
[4] Hopkins, *Journ. Path. and Bacteriol.*, Edin. and London, 1893, vol. i. p. 450.
[5] Cf. v. Jaksch, "Klinische Diagnostik," 1896, 4th edition, S. 428, 431; Ritter, *Ztschr. f. physiol. Chem.*, Strasburg, 1895, Bd. xxi. S. 288; Luff, Goulstonian Lectures, 1897, Lect. i.

Variations in the amount.—(*a*) *The relation to urea; the effects of diet.*—Variations in the quantity of uric acid have been considered from two different points of view. By some, these variations have been expressed always in relation to the quantity of urea excreted simultaneously. Such observers have felt that an increase or decrease in uric acid, which merely accompanies a corresponding change in the general nitrogenous metabolism, is of less physiological significance than a variation which occurs independently of (or out of proportion to) the latter; and since the urea excretion is a measure of this general metabolism, the uric has been, by such writers, referred to the urea output as a standard. Other and more recent authorities, seeing the origin of uric acid in an entirely distinct series of events within the body, and observing that the *urea* : *uric acid* ratio has no stable value, have recommended the entire neglect of this relation, preferring to express the uric acid output always in terms of its absolute amount.

An attempt has been made to show that urea and uric acid are always produced in the body, so as to bear a constant and definite ratio to each other, and that any alterations in this ratio indicate either a retention of uric acid on the one hand, or a sweeping out of previously retained acid on the other.[1] That this position cannot be maintained in its entirety is quite certain. Consideration of the effect of varying diet alone gives sufficient evidence against it. If the two analyses by Bunge, given in an early section of this article, be examined, we see that upon a diet of bread, not only is the absolute amount of uric acid less than upon a diet of beef, but also that the relation to urea is also strikingly less. On bread the ratio is 1 to 81, on beef it is 1 to 48. Similar results are obtained, as the writer has found, if the experiments are continued for many days. If the two substances were always produced in constant ratio we should have to conclude that a bread diet produces a continuous storing up of uric acid in the body; and for this conclusion there is certainly no evidence.

Again, if we consider the effect of varying the quantity of the ingested food—its composition being maintained uniform—we find that on the whole the uric-acid excretion is less affected by such variations than is the urea, so that we change the value of the ratio merely by altering the amount of food taken.

It is therefore impossible to look upon the ratio which uric acid bears to urea as an independent physiological constant, or to conclude that even wide variations in its value are necessarily pathological.

But some authorities go further than to say that the uric acid output is more stable than that of the urea, claiming, indeed, that it is quite unaffected by the absorption of the ordinary proteids of diet—the albumins and globulins with their derivatives. If this be a fact, and the production of the acid is independent of variations in these main nitrogenous constituents of food, we ought certainly, in studying the quantity in the urine, to neglect its ratio to urea altogether. This ratio will then be little more, under ordinary circumstances, than an expression for the urea variations, measured from the more stable uric acid output, so to speak, as a base line; while, if we are studying the effect of special factors upon uric acid production, reference to the urea will be unnecessary and misleading.

[1] Haig, "Uric Acid in Disease."

Salkowski, in 1889,[1] was among the first to give prominence to this view, but the experiments upon which he then based his opinion were not wholly calculated to decide as to what is the effect, if any, of the ingestion of ordinary proteids. They were in the main those of Hirschfeld,[2] but the experiments of this investigator were directed to a broader question than that of uric acid excretion, and the diet for the purposes of his research was made entirely abnormal, so that definite conclusions on the point we are discussing cannot be fairly drawn from them. In the experiments of Horbaczewski and Camerer,[3] undertaken with the object of ascertaining the effect of glycerin, carbohydrates, and fat, respectively, on uric acid excretion, there were certain "normal periods," in which a standard mixed diet was taken alone. The fact that the diet was carefully maintained at a uniform level makes these very careful experiments more or less unavailable for our purpose. Nevertheless, during one of these control periods, which lasted for many days, the urea excreted fluctuated somewhat widely, presumably from varying degrees of proteid absorption. Of this period the author says: "The uric acid eliminated went hand in hand with the nitrogen excretion. In general, the more the total nitrogen present the more the uric acid found."

There are but few experiments recorded which bear properly on our problem, fundamental though it be; that is to say, experiments where the uric acid and urea (or total nitrogen) have been estimated from day to day by reliable processes; while the quantity, but not the quality, of the proteids ingested has been made to vary widely. Schültze[4] found the uric acid rise with increase of flesh diet. Hester and Smith[5] found it raised when the ingestion of proteids was increased, though it was somewhat less affected than the urea. I myself have repeatedly observed a rise to follow an increase in the diet where the composition of this has been carefully maintained constant.

But these observations are open to one criticism. Whatever the effect of globulins or albumins, there appears to be no doubt that ingestion of *nucleo-proteids* increases the excretion of uric acid; calves' thymus, with its abundant nuclein, has been largely used to test this point. Umber[6] and Weintraud[7] have found that with thymus the excretion of uric acid may amount to double that of the same individual upon ordinary proteid (muscle) diet of equal nitrogenous value.

Is, then, the smaller increase found when ordinary proteid diet is taken, merely due to any nuclein present and not to the absorption of the ordinary proteids? We shall be able to add the last word to this discussion immediately.

If the effect of an *isolated meal* of ordinary mixed diet be studied, it is found that an increase in the excretion of uric acid occurs very rapidly after the food is taken. According to Marès[8] the maximum hourly excretion occurs at the fifth hour after the meal; four hours before the urea reaches its maximum. This observer held, therefore, that it was not derived directly from the ingested proteid, but from cellular activity during digestion. Horbaczewski confirmed this result, and believed that it was due to a digestive leucocytosis (*vide* article, "Metabolism"), with its consequent liberation of nucleins in the body. But Camerer[9] has recently found that this rise of

[1] *Virchow's Archiv*, 1889, Bd. cxvii. S. 572; comments on a paper by Spilker.
[2] *Ibid.*, Bd. cxiv. S. 301.
[3] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1886, Bd. xcviii. Abth. 3, S. 301.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xv. S. 427.
[5] *New York Med. Journ.*, 1892, p. 38.
[6] *Ztschr. f. klin. Med.*, Berlin, 1896, Bd. xxix. S. 174.
[7] *Berl. klin. Wchnschr.*, 1895, S. 407.
[8] *Centralbl. f. d. med. Wissensch.*, Berlin, 1888, S. 2.
[9] *Ztschr. f. Biol.*, München, 1896, Bd. xxxiii. S. 136; also Weintraud, *Chem. Centr.-Bl.*, Leipzig, 1895, Bd. ii. S. 234.

uric acid after a meal is by no means marked, unless the food taken contains nuclein. On a diet composed, for instance, of egg albumin, the rise was very small, while during the digestion of non-nitrogenous diet the output of uric acid was even diminished. Camerer holds, therefore, that digestive leucocytosis cannot be the cause of post-prandial increase, but only the actual ingestion of nucleins, and his results would suggest that we must answer the question in the previous paragraph in the affirmative, and recognise that the excretion of uric acid is not increased by the ingestion of ordinary proteids.

If this be confirmed, we must for the future attach no importance to the *urea : uric acid* ratio; and when we wish to eliminate mere dietetic effects from our study of other specific variations in the urinary uric acid (when, for instance, we are endeavouring to ascertain if retention is occurring in disease, or whether a certain drug is promoting elimination), we must do this by controlling the ingestion of nucleins during the experiments.

It should be understood, however, that in spite of much labour spent upon the problem, our knowledge of the relation of urinary uric acid to diet is scarcely yet upon a firm foundation, and contradictory statements will be found in the literature. Future investigators may have to face yet another difficulty, if it be true, as Weintraud[1] affirms, that a true excretion of uric acid may occur through the walls of the intestine.

One fact is abundantly certain—that great *individual differences* exist in uric acid excretion. In spite of all that has been said above, it is found that, with the ordinary regularity of habits and diet customary in civilised life, the uric acid output (when the whole twenty-four hours' excretion is dealt with), and even its relation to urea, will remain fairly constant in any given individual; whereas, when different individuals are compared, much greater differences are seen. Before we can say with certainty what constitutes a pathological or exceptional condition in any case, we must know the normal behaviour of the particular organisation in question (Salkowski).

The ratio borne to urea may vary in different healthy individuals from 1 : 25 to 1 : 50; the proportion most commonly found being from about 1 : 35 or 1 : 40.

(*b*) *Variations apart from diet.*—It is a well-established fact that in newly-born children the uric acid excretion bears a high proportion to the body weight, and also to the other nitrogenous constituents of the urine. In the first few days of life 7·8 per cent. of the urinary nitrogen may be in the form of uric acid.[2]

The absolute amount is increased by excessive exercise and diminished by rest. With regard to *drugs*, the action of alkalies is still disputed. It is possible that an isolated dose may temporarily accelerate excretion; but, according to Spilker and Salkowski,[3] continued administration diminishes it. There is certainly no foundation for the statements of Haig, that the excretion of uric acid varies inversely as the acidity of the urine.[4] Salicylates undoubtedly increase the amount in the urine. Pilocarpine produces an increase, possibly from the leucocytosis which follows its use. Pathologically, there is increase in

[1] *Chem. Centr.-Bl.*, Leipzig, 1895, Bd. ii. S. 310.
[2] Hofmeier, *Virchow's Archiv*, 1882, Bd. lxxxix. S. 493.
[3] Ibid., 1889, Bd. cxvii. S. 570.
[4] Cf. Herringham and Davies, also Herringham and Groves, *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. pp. 475 and 478.

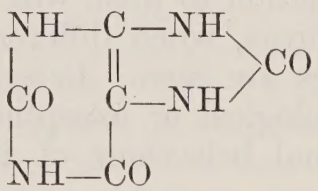
conditions of leukæmia, and this may be said to be the only well-established fact as to the effect of disease on uric acid excretion. In gout, although the urate deposits form so prominent a factor, the question of the amount excreted in the urine is still unsettled. In this country, at any rate, many cases occur in which, as originally observed by Sir A. Garrod, the excretion during the chronic condition is greatly diminished, whereas, in relation to the acute attack, increased elimination may occur.[1] Pyrexia alone does not produce any marked increase; but in certain specific fevers with a definite crisis, a large temporary increase may occur, depending, according to Horbaczewski, upon the associated leucocytosis.

Uric acid is one of the commonest constituents of urinary calculi.

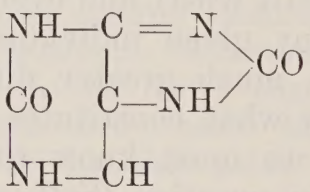
(e) **The xanthin bases.**—Several members of this chemical group are found in urine, in variable but always small amount. *Xanthin* itself was discovered by Marcet in 1819 as a constituent of a urinary calculus, and its presence in urine was first demonstrated by Strecker in 1857. In addition to xanthin, the following members of the group may be present — *heteroxanthin*, *paraxanthin*, *hypoxanthin* (*sarkin*), *guanin*, *adenin*, *and carnin*.

All these substances are closely related to each other and to uric acid; and the chemical group to which they belong also contains certain important vegetable bases.

The relation of *xanthin* to uric acid is best understood by a comparison of the structural formulæ; our knowledge of the constitution of the base being due to E. Fischer.



(uric acid)



(xanthin)

Xanthin contains one atom less oxygen than uric acid, while *hypoxanthin* contains one less than xanthin.

$C_5H_4N_4O_3$	$C_5H_4N_4O_2$	$C_5H_4N_4O$
(uric acid)	(xanthin)	(hypoxanthin)

In the laboratory means have not been found to pass from one of these three compounds to another by oxidation or reduction; but in the body the steps involving oxidation can certainly occur.

Heteroxanthin and *paraxanthin* are homologues of xanthin, the former being its methyl- and the latter its dimethyl-derivative; paraxanthin is therefore an isomer of the vegetable bases, theobromin and theophyllin.

Guanin in an imido-xanthin; that is to say, it is xanthin with an oyygen atom replaced by an NH group; and *adenin* bears the same relation to hypoxanthin.

$C_5H_4N_4O.O$	$C_5H_4N_4O.NH$	$C_5H_4N_4.O$	$C_5H_4N_4.NH$
(xanthin)	(guanin)	(hypoxanthin)	(adenin)

Uric acid and the xanthin bases are grouped together by recent

[1] Cf. Fawcett, *Guy's Hosp. Rep.*, London, 1895; Luff, Goulstonian Lectures, Lect. i.

German writers [1] under the term, "alloxuric substances," a name meant to show their relation on the one hand to alloxan,[2] and on the other to urea; the bases themselves may be designated the "alloxuric bases."

The *amount* of the xanthin bases in the urine has been generally understated until lately; they amount collectively to something like one-tenth of the uric acid present; that is to say, an average of 0·1 to 0·07 grm. of the combined bases is excreted per diem (Camerer, Salkowski). Of xanthin itself some 0·02 to 0·03 grm. is found, upon a mixed diet.

General properties.—That the xanthin compounds, unlike uric acid, are basic in character, is probably due to the fact that the CO group is absent from the central carbon chain. (Cf. graphic formulæ above.)

Their basicity is, however, very feeble, and many of their compounds with acids are decomposed by water—just as is the "sulphate" of uric acid; while, on the other hand, they are all capable of forming metallic derivatives and compounds with other bases. They contrast sharply with uric acid in their easy solubility in mineral acids. In ammonia they are also soluble (with the exception of guanin). Xanthin itself dissolves to a very slight extent in water, but the other bases are more soluble.

They are precipitated from urine—(1) By the addition of phosphotungstic or phosphomolybdic acids in acid solution; (2) by silver nitrate in ammoniacal solution; and (3) by copper salts; especially in the presence of thiosulphates. When precipitated by any of these methods, they are accompanied out of solution by uric acid (*vide infra*).

Isolation and estimation.—It is beyond the scope of this article to describe in detail the separation of the xanthin bases individually. Very large quantities of urine (100 litres and upwards) are required for the purpose. If the precipitate obtained by adding ammonia, and afterwards ammoniacal nitrate of silver solution, be decomposed by sulphuretted hydrogen, and the filtrate from the silver sulphide acidified with hydrochloric acid, concentrated, and allowed to stand, the uric acid crystallises out. This being filtered off, the liquid is again made alkaline with ammonia and the bases again precipitated with silver nitrate. The varying solubility of the silver compounds so obtained in nitric acid permits of a preliminary fractionation of the bases; and, when liberated from combination with silver, their diverse solubilities in water and other media yield methods for their final separation from each other.

If urine (100 c.c.) be heated to boiling, and precipitated with a mixed solution of copper sulphate and sodium thiosulphate, some chloride of barium being afterwards added, a precipitate is obtained which contains all the uric acid and xanthin bases, but no other nitrogenous constituent (Krüger and Wulff). By estimating the nitrogen in this precipitate by means of Kjeldahl's process, we obtain a measure of what may be called the "alloxuric nitrogen," an important urinary constant. If a separate estimation of the uric acid be made, the nitrogen proper to this may be deducted from the "alloxuric nitrogen," and we obtain a value for the "nitrogen of the bases." Such a

[1] Krüger and Wulff, *Ztschr. f. physiol. Chem.*, Strassburg, 1896, Bd. xx. S. 176.

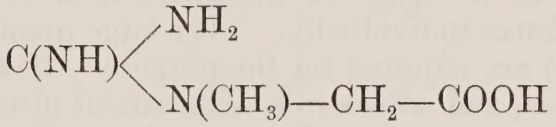
[2] Alloxan $\left\{\begin{matrix} NH—CO \\ | \quad | \\ CO \quad CO \\ | \quad | \\ NH—CO \end{matrix}\right\}$ is an oxidation product of uric acid. The xanthin bases and uric acid will all be seen to contain the urea residue and the three-carbon chain, which together comprise the so-called "alloxan ring."

procedure is our most convenient method for following the variations in the excretion of the xanthin group.[1]

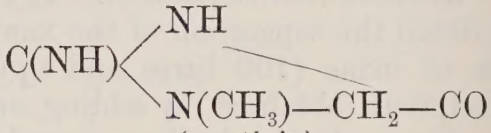
Tests.—Xanthin and its two homologues, and also carnin, but not the other bases, give *Weidel's reaction.* This is almost identical with the murexide test described for uric acid, but chlorine water is used instead of nitric acid. The bases resist oxidation with nitric acid much more fully than does uric acid, but, in the presence of a small quantity of a chloride, xanthin will give the ordinary murexide reaction. Very characteristic of xanthin and hypoxanthin are the crystalline precipitates which they yield with silver nitrate in the presence of nitric acid.

Variations in the *amount* of the urinary xanthin bases closely follow those of uric acid, and for the most part depend upon the same influences. The bases, however, are apt to vary even more widely. According to Camerer[2] they are greatly increased by certain forms of vegetable food; thus, in one experiment, on a flesh diet the nitrogen present as these bases was only 0·1 per cent. of the total nitrogen; while, when green vegetables formed the chief ingredient of the food, it was 0·6 per cent. They are increased by diet rich in nucleins,[3] and pathologically their amount is greatly raised in some forms of leukæmia.

(f) **Creatinin.**—This base is chemically distinct from the alloxuric compounds, in that its molecule contains neither the alloxan ring nor the urea residues which are characteristic of these. Nevertheless, on hydrolysis, it easily yields urea and an amido-acid (methylglycine). It is the anhydride of creatin, which is itself methylglycocyamin.



(creatin)



(creatinin)

Whether **creatin** itself is ever a urinary constituent is somewhat uncertain. It has been stated to occur when the urine is excreted in alkaline condition,[4] but the quantity is, in any case, very small. So easily are the two substances converted the one into the other, that care is requisite in the isolation of either. When creatin stands in acid solution, it tends to change into its anhydride, while creatinin in alkaline solution suffers the inverse change.

G. S. Johnson[5] has found, however, that urinary creatinin is not identical, but isomeric, with that obtained artificially from creatin (*e.g.* by the action of acids), and distinct from the creatinin found in small quantity in muscles.

The creatinin of urine was first isolated by Liebig. It is present on an average to the extent of about 1·0 grm. in the excretion of twenty-four hours, when a mixed diet is taken.

Properties.—In its compounds creatinin exhibits well-marked basic tendencies, and it can liberate ammonia from ammonium salts on boiling; but, according to Salkowski, solutions of the pure substance react

[1] For details see Krüger and Wulff, *loc. cit. supra.*
[2] *Ztschr. f. Biol.*, München, 1891, Bd. xxviii. S. 72.
[3] Weintraud, *loc. cit.*
[4] Hoffemann, *Virchow's Archiv*, 1869, Bd. xlviii. S. 358.
[5] *Proc. Roy. Soc. London*, 1892, vol. l. p. 287.

neutral to litmus. The crystalline form of the base varies with the method of preparation (Johnson). As ordinarily obtained, it exists as colourless monoclinic prisms, which are often imperfectly formed, and appear of whetstone shape (Fig. 53). It dissolves in about twelve parts of cold water, but requires a hundred parts of alcohol to dissolve it at ordinary temperatures. In ether it is almost insoluble. Creatinin reduces alkaline copper solutions (cf. p. 608). It forms characteristic crystalline salts with the mineral acids, aqueous solutions of which react acid to litmus. With certain salts of the heavy metals it forms crystalline molecular compounds, two of which are of practical importance.

Creatinin zinc-chloride—$(C_4H_7N_3O)_2ZnCl_2$—separates as a precipitate, consisting of stellate clusters of acicular crystals, when a concentrated neutral solution of chloride of zinc is added to an aqueous or alcoholic solution of the base. The compound is soluble in hot water, in mineral acids, and in alkalies; but insoluble in alcohol, and very slightly soluble in cold water.

Creatinin mercuric-chloride, a complex compound of the formula $4(C_4H_7N_3O.HCl.HgO),3HgCl_2$. This is precipitated in colourless, glassy, spherular masses, when sodium acetate and mercuric chloride are added to creatinin solutions. The base is also precipitated, even from very dilute solutions, by the addition of phosphotungstic, phosphomolybdic, or picric acids.

Isolation and estimation.—Neubauer separated creatinin from the urine by means of its combination with zinc chloride, this salt being added to an alcoholic extract of the evaporated urine. A more convenient method is to treat the urine direct with a little sodium acetate, and then with one-fourth its volume of saturated mercuric-chloride solution. The precipitate which first falls is at once filtered off; it contains uric acid and other constituents, but not creatinin. The filtrate from this rapidly begins to deposit the mercury compound described above, and in forty-eight hours precipitation is complete (G. S. Johnson). The base itself is prepared by decomposing this precipitate with sulphuretted hydrogen, and by treating the creatinin-hydrochloride, so obtained, with hydrate of lead.[1] To determine the quantity, the mercury precipitate may itself be weighed, and the percentage of creatinin calculated from this.[2]

Tests.—If a solution of creatinin be treated with a small quantity of very dilute sodium nitroprusside solution, and subsequently with weak caustic alkali, a rich, ruby-red colour is produced, which afterwards changes to yellow (*Weyl's reaction*). If acetic acid be now added in excess, and heat applied, the solution becomes green, and then blue, and finally a precipitate of Prussian blue is formed. Acetone (p. 616) gives an analogous reaction, but behaves differently after the addition of the acetic acid. Many specimens of urine will give Weyl's test direct.

Jaffé's test is an application of the fact that creatinin gives, with picric acid and caustic alkali, an intense red colour, even in the cold.

The *variations* in the urinary creatinin generally follow very closely those of the urea, but there can be no doubt that its quantity depends largely on the amount of creatin taken with the food. Its physiological relations are discussed elsewhere. Pathologically, it is increased in most febrile conditions, and in diabetes. It has been stated to diminish in

[1] In this process all the operations are carried out in the cold; by this means the true urinary creatinin is obtained. Heat produces isomeric change.

[2] Cf., however, Allen, "Chemistry of Urine," pp. 156 and 159.

progressive muscular atrophy, and in pseudo-hypertrophic paralysis. According to Senator, no increase is produced by the paroxysms of tetanus—a fact which is of interest as bearing on the relation of muscular activity to the urinary creatinin.

(g) **Hippuric acid.** — *Hippuric acid* is benzamido-acetic acid, or benzoylglycin, $C_6H_5.CO.NH.CH_2.COOH$; in other words, it is a condensation product of benzoic and amido-acetic acids, in the formation of which the hydroxyl group of the former is eliminated as water, with an atom of hydrogen from the amido group of the latter. But

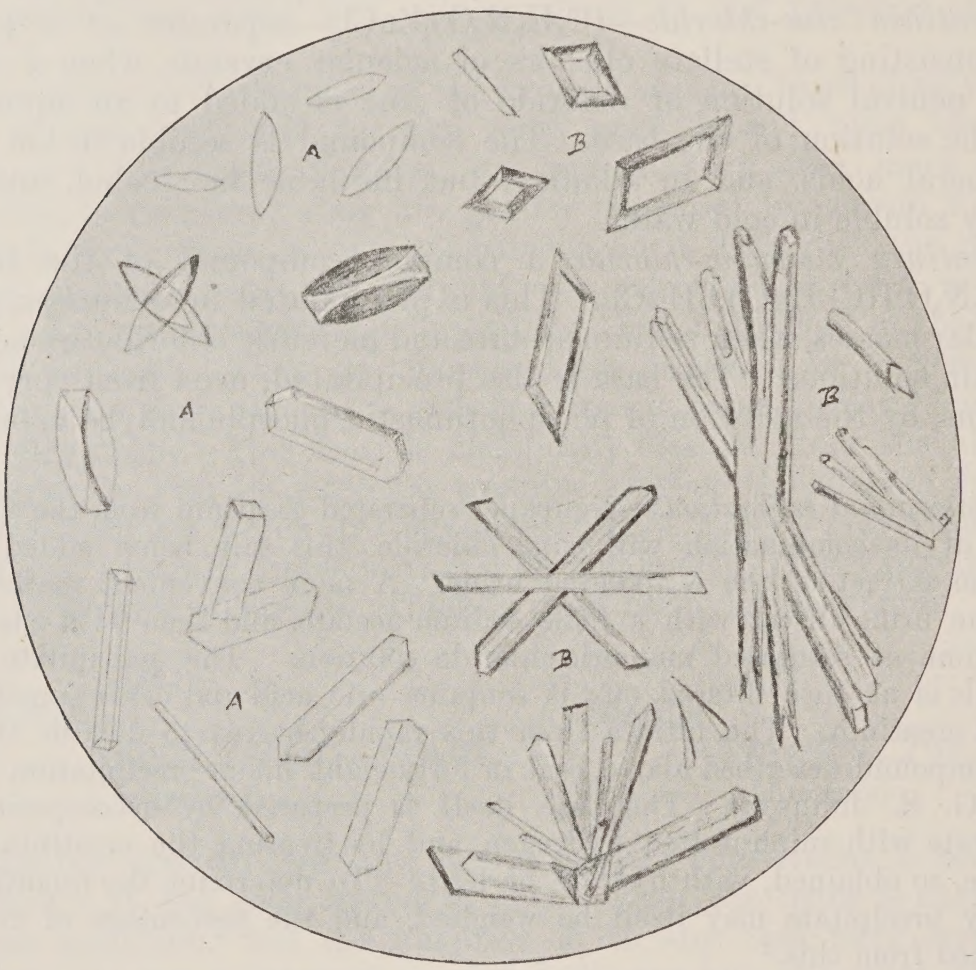


FIG. 53.—A. Creatinin; B. Hippuric acid.

the simplest artificial synthesis is obtained when monochlor-acetic acid is heated with benzamide.

$$C_6H_5CO.NH_2+CH_2Cl.COOH=(C_6H_5.CO)NH.CH_2.COOH+HCl$$

In most mammals the synthesis by dehydrolysis occurs in the kidney; hippuric acid appearing in the urine, whenever benzoic acid, or precursors of benzoic acid, are taken by the mouth. The excretion of hippuric acid is, indeed, mainly dependent upon the relative richness of the diet in such precursors of benzoic acid.

It is not necessary that benzoic acid should itself be ingested. A benzene derivative containing a single "side-chain" is nearly always oxidised in the body to benzoic acid. Such substances, therefore, as toluene, $C_6H_5.CH_3$; cinnamic acid, $C_6H_5.CH.OH.COOH$; or phenyl-propionic acid, $C_6H_5.CH_2.CH_2.COOH$, all give rise to an excretion of hippuric acid when they are taken by the mouth. Aromatic compounds of this type are abundantly present in some forms of vegetable food, as

in many fruits and in the cortical parts of most plants. Vegetable food greatly increases, therefore, the excretion of hippuric acid.

But the vegetable aromatic compounds are not the sole source of the urinary hippuric acid. In the decomposition of proteids, which occurs in the bowel, aromatic residues split off. Precursors of benzoic acid (mainly, perhaps, phenylpropionic acid) are thus formed, and after oxidation they appear in the urine as hippuric acid. The metabolism of the tissue proteids themselves, moreover, may yield precursors of the same kind, so that even in starvation hippuric acid does not wholly disappear from the urine.

This dual origin (from aromatic precursors in the diet chiefly, but likewise from proteid metabolism) is found also in the case of the other aromatic constituents of the urine (p. 605).

Upon a mixed diet the excretion of hippuric acid in human urine amounts to about 0·7 grms. per diem; upon a diet rich in fruits it may be raised to three or four times this. In herbivora the quantity is much larger; the urine of cattle, for instance, often contains as much as 2 per cent., though, as might be expected, that of sucking calves only contains small amounts.

Properties.—It forms four-sided prismatic crystals ending in two or four facets, and often grouped in clumps (Fig. 53), of which the melting point is about 187°. It is but slightly soluble in cold water or alcohol; but both these solvents dissolve it easily when hot. It is soluble in acetic ether, but not so in most other organic liquids. If heated to 240° it decomposes, benzoic acid subliming out and a reddish residue being left behind. When first heated at this temperature a hay-like odour is given off, which is succeeded by that of prussic acid. When boiled with strong hydrochloric acid it splits up into its components, benzoic and amidoacetic acids. The growth of the *Micrococcus ureæ* can bring about this decomposition, so that stale specimens of urine often contain benzoic in place of hippuric acid. Taken to dryness with nitric acid, it yields an odour of nitrobenzene.

Solutions of hippuric acid react acid to litmus, and even when very dilute they impart a violet colour to congo-red. By the use of the latter indicator Brücke proved the absence of the free acid from the urine. It is present always as salts. It forms salts with bases, but does not combine with acids. Its iron compound is insoluble in hot water, and may be employed in separating the acid from its solutions.

Isolation and estimation.—The method of Bunge and Schmiedeberg[1] consists in making an alcoholic extract of the urinary solids, evaporating off the spirit, dissolving the residue in water, and, after acidifying with hydrochloric acid, shaking up repeatedly with successive quantities of acetic-ether. On evaporating the latter, impure crystals of the acid are obtained, the impurities being removed by treatment with petroleum-ether, in which hippuric acid is insoluble.

Tests.—The substance is recognised by its crystalline form, by its melting point, by its behaviour on heating, and by the formation of its insoluble iron compound when neutral ferric chloride is added to its solutions.

In addition to hippuric acid, minute quantities of its homologue *phenaceturic acid* (phenylacetylglycin), $C_6H_5.CH_2CO—NH.CH_2.COOH$,

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. vi. S. 235.

are occasionally found in human urine. Its origin and significance are analogous to those of the more abundant substance.

When benzoic acid or its precursors are administered to birds, they are excreted as *ornithuric acid*, which is an analogous conjugated compound of benzoic acid with diamidovalerianic acid.

(h) **Amido-acids.**—These, in simple unconjugated form, are seldom found in normal urine. Under certain pathological conditions *leucine* and *tyrosine* appear in considerable quantities. The elimination of these substances is especially associated with conditions in which a rapid destruction of the hepatic tissue has occurred; thus they are found in acute yellow atrophy of the liver, and, to a less extent, in phosphorus-poisoning.

When these amido-acids are given by the mouth in moderate quantity, and under conditions of normal health, their nitrogen is excreted wholly in the form of urea. If, however, tyrosine be administered in very large amounts, it may be excreted in part as tyrosine-hydantoin, in which it exists as a conjugate compound with urea;[1] and at the same time other aromatic constituents of the urine are increased in quantity by derivation from its aromatic nucleus. Only when the normal hepatic functions are in abeyance does the unaltered amido-acid itself appear.[2]

When present in urine, leucine and tyrosine are usually found together. If in large quantity, they may, though very rarely, form a deposit; at other times they may be seen under the microscope when a drop of the urine is evaporated.

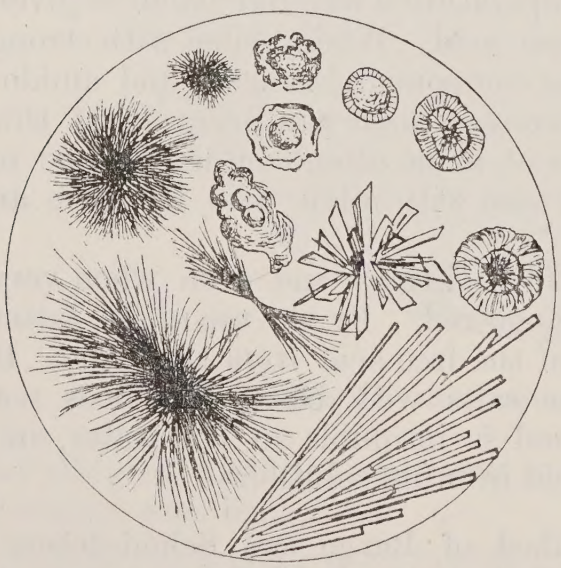


Fig. 54.—Leucine and Tyrosine.

In general, however, they must be separated by special means. The leucine may be dissolved, by means of hot alcohol, from the residue obtained by evaporating the urine, and when the alcoholic extract cools it separates as a greasy mass, which under the microscope will be seen to consist of minute spheroids with concentric markings interrupting a radiated structure. To demonstrate the presence of tyrosine, the urine is first precipitated with basic acetate of lead, the filtrate from the lead precipitate treated with sulphuretted hydrogen and again filtered. On thorough concentration and cooling of the lead-free filtrate, the tyrosine separates out in characteristic acicular prisms, which are mostly combined into sheaves or stars (Fig. 54).

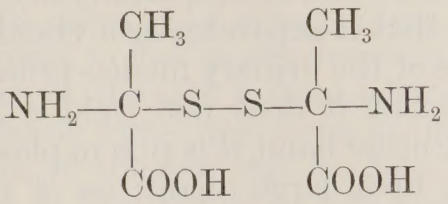
Cystine [3] is another amido-acid, but it is at the same time a sulphur-containing substance, differing in its metabolic significance from leucine and tyrosine.

[1] Jaffé, *Ztschr. f. physiol. Chem.*, Strassburg, 1883, Bd. vii. S. 306.

[2] According to the recent observations of Ulrich, leucine and tyrosine are always to be found in normal urine, though in small quantity, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1897, Bd. xi. S. 12.

[3] Cf. Baumann, *Ztschr. f. physiol. Chem.*, Strassburg, 1884, Bd. viii. S. 299; also Brensinger, *ibid.*, 1892, Bd. xvi. S. 552.

It is a sulphur derivative of an amidolactic-acid, and has the formula:



It may appear in small quantity in certain diseases, but is generally a product of peculiar disordered metabolism, which is found to be characteristic of certain families. Members of such families may excrete habitually from 0·5 to 1 grm. daily. It sometimes separates as a crystalline deposit from the urine, and occasionally forms calculi in the urinary tract.

Physiologically it is of interest, in that cystine or substances allied to it are probably the precursors of certain of the normal sulphur compounds of the urine (p. 632).[1]

Its crystals are very characteristic, being usually in the form of hexagonal plates (Fig. 55); more rarely it appears in rhombohedral form. Urine which contains it will, if heated with caustic potash and plumbic acetate, give a black precipitate of lead sulphide.

Proteids.

Normal urine contains but traces of substances belonging or allied to the proteid group. But minute quantities of a *nucleo-proteid* derived from the cells of the urinary passages are seldom or never absent. In the majority of cases the amount of this is so small that it is difficult directly to demonstrate its presence. The flocculent cloud which generally separates on standing, even from the clearest urine, by no means always contains any isolated proteid, but may consist entirely of intact epithelium cells. But the nucleo-proteid may be detected by suitable tests in the precipitate which falls when large quantities of normal urine are mixed with alcohol.

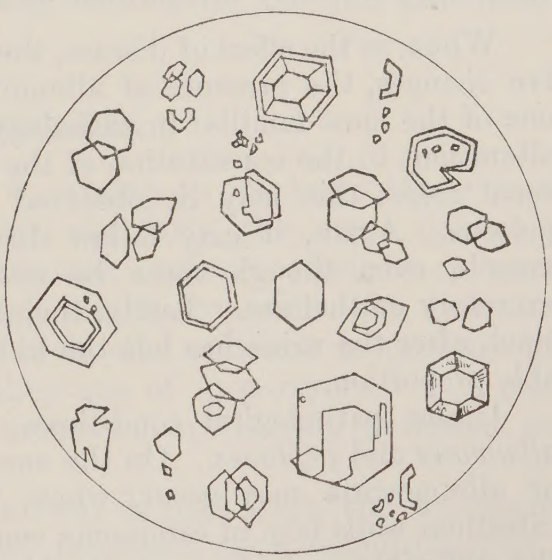


Fig. 55.—Cystine.

The nucleo-proteid may, on the other hand, so far increase in conditions of apparent health, that the urine will react to Heller's test (*vide infra*). Thus Flensburg[2] found, on examining the urine of 1252 healthy persons, that 97 of these gave a reaction with nitric acid, which could be shown to be due to a nucleo-proteid.

In such cases, and in others where the increase is greater and due to inflammatory changes in the urinary tract, the nucleo-proteid may be precipitated by the addition to the urine of acetic acid in the cold; especially if the fluid be first diluted to eliminate the solvent action of

[1] Goldmann and Baumann, *ibid.*, 1888, Bd. xii. S. 254.
[2] *Skandin. Arch. f. Physiol.*, Leipzig, 1893, Bd. iv. S. 410.

the salts present, or, better still, if the salts be first reduced by dialysis. In some pathological conditions, and especially in cystitis, the amount may be so greatly increased that it separates as a viscid gelatinous precipitate. The mucoid appearance of the urinary nucleo-proteid led to its being long looked upon as mucin; but it does not yield a reducing substance on hydrolysis, while, on the other hand, it is rich in phosphorus. Nevertheless, recent researches made upon large quantities of urine indicate that the precipitate given by acetic acid contains small quantities of ordinary *mucin*, or a phosphorus-free mucoid, as well as the nucleo-proteid.[1]

Apart from increase due to inflammatory conditions of the excretory tract, nucleo-proteid is said to be increased when the blood is exceptionally rich in leucocytes (leukæmia).[2]

The question as to whether or not normal urine contains *serum albumin* or *serum globulin* offers a problem of the same order as that of physiological glycosuria, fully discussed on p. 608. The matter is, however, of less importance physiologically than is the latter question, as, although the evidence to hand points to the fact that if sufficient urine is employed these proteids may nearly always be separated in minimal traces, it by no means follows that they form part of the true excretion, for they may arise rather, like the nucleo-albumin, from the surface of the urinary tract.

As to the cases when, in apparent health, there is such an increase of these proteids that their presence may be shown by the direct application of ordinary tests, we are met with the difficulty of having to define what is meant by "normal" urine. Such quantities may be present, for instance, after exceptionally severe exercise, as in the urine of soldiers after prolonged marching (Leube, Chateauburg); but it is not certain that the excretory mechanism is here working physiologically.

When, as the effect of disease, the renal epithelium has undergone degenerative changes, the presence of albumin in the urine is a common phenomenon; one of the most familiar in pathology. Albuminuria may arise, too, from such alterations in the constitution of the blood as upset its normal relations to the renal cells; this may be observed in anæmia, and as the effect of specific poisons. Again, it may follow disturbances of blood pressure in the renal vessels, even though these be unassociated with obvious changes in the excretory epithelium. Lastly, the albumin due to addition from the excretory tract, after the urine has left the kidneys, may pathologically reach a considerable proportion.

Under pathological conditions, also, the urine may come to contain *albumoses* and *peptones*. On the one hand, a so-called enterogenous peptonuria or albumosuria may occur, when, from degenerative changes in the gastro-intestinal walls (*e.g.* in carcinoma ventriculi or the ulcerative stage of enterica), the diffusible proteids reach the blood stream and thereupon are immediately eliminated by the kidneys. On the other hand, these substances may reach the blood stream from abscesses or other purulent collections where the tissue proteids have been hydrolysed by the growth of organisms. Whatever their origin (and it is sometimes not so clear as in the above groups of cases), the proteoses and peptones no sooner reach the blood than they are found in the urine. The older methods of investigation did not clearly distinguish between peptones and albumoses in the urine; evidence is now accumulating to show that the latter are by far the more common.

[1] Cf. Malfatti, *Wien. klin. Wchnschr.*, 1891, S. 433; also Mörner, *Skandin. Arch. f. Physiol.*, Leipzig, 1895, S. 437.

[2] According to recent observations, the nucleo-proteid of urine is in some cases to be identified with Lilienfeld's "nucleohiston."

A very large number of *tests* for the presence of albumin and globulin in the urine have been described. We can here refer to two only.

Heller's test.—A small quantity of strong nitric acid is placed in a test tube, and the urine is allowed to flow gently down the side of the tube so that it floats upon the surface of the acid without mixing with it. If coagulable proteids are present, a dense white ring forms at the junction of the liquids. As little as ·002 per cent. of albumin may be thus detected. The urinary nucleo-albumin may react to this test if in sufficient quantity, but the ring formed is less dense, and more apt to be formed at some little distance from the acid.

Ferrocyanide test.—A solution of potassic ferrocyanide is first added to the urine, and the mixture made acid with acetic acid, when the albumin and globulin are precipitated as a flocculent cloud. If the salt be added before the acid, nucleo-proteid is not precipitated.

To separate serum globulin from albumin, the urine is, after neutralisation, saturated with magnesium sulphate, which precipitates the former. The precipitate may contain certain of the salts of the urine, and heteroalbumose if present. The proportion of globulin to albumin may vary greatly, and may be quite different from that present in the blood.

To detect peptones, the urine is saturated with sulphate of ammonium, and, after standing, filtered; the biuret test may now be applied to the filtrate, a large excess of caustic alkali being used. The ammonium sulphate precipitate contains (in addition to ammonium urate) all other proteids present and also the urinary mucin. If this precipitate be allowed to stand under alcohol for some days, the proteoses are obtained in solution when it is extracted with water. The presence of urates must be borne in mind when the ammonium-sulphate precipitate is being dealt with, as these yield a coloration with certain proteid tests; and again, may lead to error if ordinary mucin is to be identified by its yield of a reducing body on boiling with acids, for uric acid itself reduces copper solutions.

Aromatic Substances.

In addition to hippuric acid, which, owing to its importance as a nitrogenous constituent, we have treated specially, the urine contains other substances belonging to the aromatic group; that is to say, substances the molecular structure of which contains the benzene nucleus. Under normal circumstances each one of these is present in very small amount, but collectively they are of importance. For our knowledge of their chemistry in the urine we are largely indebted to the initiative work of Baumann.

Like hippuric acid (*q.v.*), they are derived in part from the aromatic constituents of the food, and they are all increased by a vegetable diet; but also, like hippuric acid, they partly arise from the breakdown of proteids. In their derivation from the latter it is possible that tyrosine in the bowel is an intermediate stage, as many of them are greatly increased when that substance is given by the mouth.[1] We cannot deal with these substances in great detail, but the characteristic types of compounds in which the aromatic nucleus is found in the urine should be noted. *They comprise, mainly, simple hydroxyl-substitution products of benzene, and carboxyacids related to these.* We shall also consider in this section urinary indol and skatol, which are nitrogenous aromatic compounds. Most of the substances to be dealt with are

[1] Brieger, *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. ii. S. 256; Wolkow und Baumann, *ibid*, 1891, Bd. xv. S. 228.

excreted as *conjugated sulphates* (see p. 631), but the carboxyacids are only in part so excreted. Inosit, which belongs to this group chemically, has somewhat different physiological relationships to the other aromatics of urine.

Phenol ($C_6H_5.OH$) and kresol ($CH_3.C_6H_4.OH$).—Traces of carbolic acid are present in urine physiologically, but the "phenol" of the normal fluid consists, as a matter of fact, mainly of the homologous *kresol*; and, of the isomeric forms of the latter, *parakresol* is the commonest. The properties of this substance, however, closely resemble those of phenol itself. The amount of phenol and kresol taken together may be upon a mixed diet no more than some 30 mgrms. per diem. If the urine be acidified and distilled, the distillate made alkaline, concentrated, and, after concentration, neutralised, on the addition of bromine a whitish precipitate will appear, due mainly to the formation of the tribromphenols.

Pyrocatechin (orthodihydroxybenzene) and hydrochinon (paradihydroxybenzene) $C_6H_4(OH)_2$.—Of these two isomeric substances the former is a constant constituent of human urine in small quantity; the latter is found probably only under exceptional circumstances. The former is easily removed from the acidified urine by shaking with ether; but its subsequent purification involves a lengthy procedure.[1] It is a white crystalline volatile solid, easily soluble in water. It gives a dark green coloration with ferric chloride, which, on the addition of ammonia, becomes violet and afterwards cherry-red.

Inosit.—This substance, from its sweet taste, was originally classed with the sugars, and was known as "muscle sugar." It strictly belongs, however, to the group of substances we are considering, as it is by composition hexahydroxybenzene $(CH.OH)_6$. It appears in normal urine with considerable frequency when polyuria is induced by diuretics or by copious drinking. On the other hand, its appearance is not entirely dependent upon the flushing of the tissues which such polyuria might denote, as extreme polyuria is at other times not associated with inosituria. It may occur in diabetes. Galloise found it in five out of thirty cases.

It may be separated from the urine by precipitation with acetate of lead. The precipitate is decomposed with hydrogen sulphide, the fluid concentrated, and finally precipitated by admixture with a large bulk of alcohol. The alcohol precipitate is dissolved in water, the solution mixed with an equal bulk of spirit and poured into ether, which precipitates the inosit almost pure.

The substance forms crystals not unlike those of cholesterin. It is optically inactive and does not ferment. It is said to yield sarcolactic acid by the action of bacteria.

Of the **aromatic carboxyacids** the following have been identified in human urine:—*Parahydroxyphenyl-acetic acid* $OH.C_6H_4—CH_2.COOH$; *parahydroxyphenyl-propionic acid* $OH.C_6H_4—C_2H_4.COOH$; *dihydroxyphenyl-acetic acid* $(OH)_2—CH_2.COOH$ (the homogentisic acid of Wolkow and Baumann);[2] and *trihydroxyphenyl-propionic acid* $(OH)_3C_6H_2.C_2H_4.COOH$ (the uroleucic acid of Kirk).

In the urine of herbivora other analogous compounds have been

[1] Cf. Halliburton, "Chemical Physiology and Pathology," 1891, p. 745.

[2] *Loc. cit.*, 1891, Bd. xv. S. 241. This is that isomeric acid which is related to hydrochinon.

detected, and *kynurenic acid*, a related substance, is an important constituent of dog's urine.[1]

Pathologically, the substances just described may become of considerable importance. In carbolic acid poisoning many of them are excreted in greatly increased amount; pyrocatechin and hydrochinon may be present in large quantity, and then give rise, by their oxidation, to the peculiar coloration seen in *carboluria*. In certain diseases other members of the group are increased, and give rise to the phenomena of *alcaptonuria*. In this state the urine develops, on standing, a dark colour, like that seen in carboluria; and Bœdecker in 1861 isolated a substance which he termed alkapton, to the oxidation of which he held the colour due. Alkapton was shown by Marshall and Kirk to be impure uroleucic acid (*vide supra*). This latter substance, however, is not wholly responsible for the coloration phenomenon. Wolkow and Baumann[2] have recently shown that in a case investigated by them the "alkaptonuria" was almost wholly due to the presence of homogentisic acid (*supra*). Pyrocatechin is doubtless sometimes the cause.[3] Most hydroxy-derivatives of benzene in alkaline solution develop a dark coloration on exposure to the air[4] (cf. p. 630).

The quantity of phenol and kresol in the urine is increased in extreme constipation, in obstruction of the lower bowel, in peritonitis, and in pyæmia (cf. indoxyl, *infra*, and p. 631).

Indoxyl and skatoxyl.—These, although nitrogenous compounds, are closely related to the substances just treated, and may fitly be considered here.

Indoxyl ($C_6H_4.NH.CH.C.OH$).—The so-called urinary indican is indoxylsulphuric acid. In normal urine on a mixed diet, the quantity present is only from 5 to 20 mgrms. In herbivora the quantity is much larger. It is absent from the urine of new-born children (Senator). Indoxyl is derived from oxidation in the body of the *indol* absorbed from the bowel, and its amount is increased, like that of the urinary phenols, by all causes which lead to increased bacterial decomposition of proteids, in the intestine or elsewhere; and by circumstances which favour the absorption of the indol when formed (intestinal obstruction, etc.). *Skatoxyl* (C_9H_8NOH) is derived from skatol (methyl-indol), and accompanies indoxyl into the urine by parallel paths and from kindred causes. Like indoxyl, it is present as a conjugated sulphate.

By oxidation indoxyl forms indigo-blue and indigo-red, while skatoxyl similarly yields red pigments. The consequent colour phenomena which arise in the urine are discussed under the head of the pigments.

Carbohydrates and Related Substances.

Normal urine contains small quantities of certain carbohydrates. Under ordinary circumstances the physiological limit extends only to a minute quantity of any one of these substances; but, in the urine of women during lactation, milk-sugar may occur in very considerable amount, without departure from what must be considered physiological conditions.

[1] For phenaceturic acid, see under hippuric acid.

[2] *Loc. cit.*

[3] Cf. v. Jaksch, "Klinische Diagnostik," 4th edition, 1896, p. 415.

[4] The behaviour of the alkaline solution of pyrogallic acid used in photography will be an example familiar to many.

The general chemistry of the carbohydrates is elsewhere discussed. We shall deal only with their relation to the urine:—

(a) **Dextrose.**—The question as to whether or not small amounts of grape-sugar are excreted in the urine during normal health has been much debated. It is needless to confuse the issue by an attempt to define what is meant by "normal" urine. We may ask rather, Does the urine of the average individual, living an ordinary life, upon ordinary diet, generally contain sugar? There can be little doubt, in the light of our present knowledge, that this question must be answered in the affirmative.

Brücke [1] was the first (in 1858) to state that sugar is normally present in human urine, and Bence Jones [2] was an early supporter of the view. For some years, however, the question was treated as an open one, and in 1871, Seegen, after careful study of the matter, decided that means were not then to hand by which its presence could be proved with certainty. Pavy,[3] in 1878, affirmed that it was certainly a normal constituent, and has always maintained this position. Since then other observers (in England especially Sir G. Johnson and G. Stillingfleet Johnson [4]) have stoutly maintained the contrary. The chief criticism of the earlier methods of demonstration which gave positive results was that, while they depended upon reduction tests, they did not eliminate the influence of other reducing substances. The creatinin, uric acid, hippuric acid, and other aromatic constituents of the urine, all tend to reduce salts of the heavy metals in alkaline solution. It is admitted by all observers that normal urine exercises a reducing power on copper solutions, which, if due to glucose, would indicate the presence of about 0·1 to 0·3 per cent. of this substance. But it is equally admitted by all that a large part of this reduction is due to the other substances mentioned above. The question which has been at issue is as to whether any part of the reducing power is due to sugar.

It is evident that we cannot rely alone upon reduction tests applied to the original urine. The more accurate knowledge that we now posesss with regard to the question has been obtained by three lines of investigation:—(1) By the application of direct tests which are unaffected by substances other than sugar; (2) by the use of methods which involve a preliminary removal of interfering substances; and (3) by the employment of means whereby the sugar itself is separated from the urine unmixed with the constituents which lead to error.

1. *The phenylhydrazine test of Fischer and v. Jaksch* has given positive results in the hands of several observers when applied directly to normal urine.[5] The yellow crystals of phenylglucosazone may certainly be obtained from urine containing as little as 0·1 per cent. of sugar (v. Jaksch). In my own experience great care is generally necessary to secure unequivocal results in the case of normal urine. As a crucial test, it suffers the disadvantage of yielding crystals with the glycuronic acid compounds; the amount of crystals obtainable from normal urine direct being in general too small for discriminating tests to be applied to them. After the sugar has been previously isolated, the reaction with phenylhydrazine is, however, of the utmost value as a confirmatory test (*vide infra*).

A colour reaction may be observed in normal urine, which is held by some to be conclusive of the presence of sugar. This is the *furfurol reaction.* A

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1858, Bd. xxix. S. 346.
[2] *Journ. Chem. Soc.*, London, vol. xiv. p. 22.
[3] *Guy's Hosp. Rep.*, London, vol. xxi. p. 413.
[4] See articles and correspondence in the *Lancet*, London, during July and August 1894.
[5] Cf. E. Roos, *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 523; A. H. Allen, "Chemistry of the Urine," 1895, p. 89.

small quantity of β-naphthol dissolved in chloroform is added, and then some strong sulphuric acid. The latter, by acting upon the traces of sugar present, produces furfurol, which, with the β-naphthol, gives a violet or carmine-red coloration.[1] This test is also affected by the presence of glycuronic acid.

2. By treating the urine with mercuric acetate, creatinin and the various non-saccharine reducing substances are precipitated. G. S. Johnson has maintained that in the filtrate obtained after treating a normal urine in this way, no sugar reaction can be observed. A. H. Allen has, however, obtained positive results.[2]

3. By far the most satisfactory evidence is obtained by methods capable of isolating any sugar that may be present. Moritz, by treating 5 to 6 litres of the urine of healthy men with lead salts and ammonia, and by decomposing the precipitate so obtained with sulphuretted hydrogen (Brücke's method), was able to isolate a substance which gave all the reactions of grape-sugar. It was fermentable with yeast, yielded phenylglucosazone crystals, was dextrorotatory, and reduced alkaline copper and bismuth solutions.[3] Pavy, by a similar method, long ago obtained a fermentable reducing body from normal urine, and he has since extended his earlier results by showing that the substance yields phenylglucosazone.[4]

When solutions of carbohydrates are treated with benzoylchloride, they yield a precipitate of insoluble compounds (esters) with benzoic acid. Glycuronic acid gives no precipitate. Baumann has applied this fact to the separation of urinary carbohydrates; and, in the hands of Wedenski[5] and Baisch,[6] the method has yielded very convincing results. The last observer decomposed the benzoic esters he obtained from normal urine, with alcoholic soda, and isolated, *inter alia*, a sugar which gave, with phenylhydrazine, an osazone melting at the right temperature for that of glucose. The product gave also all the other reactions of dextrose. The quantity found varied from 0·08 to 0·18 grms. in the twenty-four hours.

The evidence we have detailed leaves little room for doubt that grape-sugar is a constituent of normal urine, and we may take the figures just quoted from Baisch as the most accurate estimate we possess of its amount. Pavy and v. Udránsky found larger quantities, and Seegen considerably less, but their methods are perhaps more open to question from the quantitative point of view.[7]

Alimentary glycosuria.—It is certain that many healthy individuals, after a meal rich in sugar, and especially after the consumption of an excessive amount of sugar in solution—as in sweet wines and the like—excrete temporarily quantities of sugar greatly in excess of the small normal constant we have just discussed. The explanation of this is probably to be found in the observation of Ginsberg,[8] that when large quantities of sugar are present

[1] Molisch, *Centralbl. f. d. med. Wissensch.*, Berlin, 1888, Nos. 34 and 49. Also Luther, *Chem. Centr.-Bl.*, Leipzig, 1891, Bd. ii. S. 90; v. Udránsky, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 380.

[2] *Loc. cit.*, p. 19.

[3] *Deutsches Arch. f. klin. Med.*, Leipzig, 1890, Bd. xlvi. S. 252. A complete review of the earlier literature will be found in this paper.

[4] "Physiology of the Carbohydrates," 1894, p. 180 *et seq.*

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 122.

[6] *Ibid.*, 1894, Bd. xviii. S. 193; 1895, xix. S. 348; xx. S. 249.

[7] For a criticism of Brücke's lead-precipitation method, see Colls, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 109.

[8] *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xliv. S. 306.

in the bowel, some may be absorbed, not by the ordinary path of the capillaries and portal vessels, but by way of the lacteals and thoracic duct, thus escaping the influence of the liver. The percentage of sugar in the blood is thereby increased, and the excess is excreted by the kidneys. A distinction between such cases and those in which diabetes exists, is seen in the fact that the "alimentary glycosuria" is not produced by starchy foods, however large the quantity taken, but only by excess of ready-formed sugar in the diet.[1] According to Moritz, dextrose, lævulose, cane-sugar, and probably milk-sugar, may all appear unaltered in the urine when severally taken by the mouth in considerable quantity (*e.g.* 200 grms.). Such alimentary effects last from three to six hours.[2]

Pathological glycosuria.—The great increase of dextrose in the urine of diabetes is a familiar phenomenon. Its excretion may range in this disease from quite small quantities up to 500 or 600 grms. per diem. The morning urine is apt to contain least sugar; that passed three or four hours after a meal generally contains most.

In other diseased conditions, quite apart from diabetes, a special tendency has been observed to the occurrence of an alimentary glycosuria; gout, exophthalmic goitre, and certain nervous diseases may be instanced. An increase of reducing substances, almost certainly consisting at least in part of dextrose, is said to be found in the urine of some pyæmic conditions. As the effect of certain drugs and toxic substances, such as chloral, chloral amide, morphine, hydrocyanic acid, turpentine, and carbon monoxide, the urine commonly reduces copper solutions; but in most of these cases the reduction is due to conjugated compounds of glycuronic acid, and not to dextrose (*vide* p. 613).

The *detection* and *estimation* of dextrose, which, as we have seen, have proved difficult problems in the case of the minute amount normally present in urine, become easy when the increased amount excreted in disease is to be dealt with. The methods used depend upon the reducing power which the sugar exerts upon metallic salts, or upon certain coloured organic substances, and these may be checked by the fermentation of the suspected urine by means of yeast, by the indications of the phenylhydrazine test, and again by the use of the polariscope. Of reduction tests a great number have been proposed; we shall here refer to two only. The well-known *Fehling's test* consists of a solution of copper sulphate of definite strength, mixed with caustic alkali and alkaline tartrates (Rochelle salt). The presence of the last prevents the precipitation of cupric oxide when the solution is boiled by itself, but allows the precipitation of yellow or cuprous oxide when reduction has occurred from the action of the sugar. The reduction may be observed, after boiling the liquid, if the urine contain not less than 0·2 per cent. of dextrose. If less than about 0·5 per cent. be present, no precipitation occurs until after cooling, when the liquid becomes opaque, and of a greenish colour. With larger amounts a definite precipitate of a yellow or red colour is seen, immediately after heating the test with a small proportion of the urine. *Nylander's solution* has some advantages over Fehling's, in that it is much less affected by creatinin, urates, and reducing bodies other than sugar (*vide supra*). It is a modification of the bismuth test, originally suggested by Böttcher, and is prepared with the same reagents as Fehling's solution, but with the substitution of basic nitrate of bismuth for the copper sulphate. On boiling this solution with urine containing sugar, the liquid turns black. The reaction is easily seen if 0·1 per cent. or upwards of dextrose is present.

Both solutions are reduced by lactose and by glycuronic acid; but the former of these substances can only be present under special circumstances

[1] Cf. Neumeister, "Lehrbuch der physiol. Chem.," Th. 2, S. 306.
[2] Moritz, *Centralbl. f. klin. Med.*, Bonn, Bd. xii. No. 28.

(*infra*), while the latter is never present in the urine in amounts large enough to lead to confusion with glycosuria in the pathological sense; except when quite special substances have been taken by the mouth. But in order to make the identification of glucose more certain, we may confirm the results of a reduction test by means of *yeast fermentation*. Lactose and glycuronic acid do not ferment. The urine should be placed in a test-tube so as completely to fill it, and the tube inverted over a basin containing a further quantity of the urine. After ascertaining that no air is present, a small piece of pressed yeast is passed under the inverted tube, and the latter secured in position with a clamp. The tube is then allowed to stand at a temperature of 25° to 30° C. In twelve hours, if dextrose be present in quantity, a notable amount of carbon dioxide will have collected in the upper part of the tube. The fermentation test is very conclusive, but it is not easily obtained when less than 0·5 per cent. dextrose is present. With phenylhydrazine, however, as already stated, urines containing as little as 0·1 per cent. will yield easily recognisable crystals of phenylglucosazone. The fact that lævulose also ferments with yeast, and yields an identical glucosazone, is of little importance in practice; this sugar is rarely present (*infra*), and except under special circumstances it is unnecessary to distinguish it from dextrose.

For the estimation of dextrose, modifications of the various tests just described are employed. We may ascertain, for instance, how much of a given specimen of urine is required to precipitate all the copper from a measured quantity of standardised Fehling solution. Or, with greater convenience, we may employ the modified copper test known as Pavy's solution. This contains a large excess of ammonia, in addition to the ordinary constituents of Fehling's test. In ammoniacal solution cupric salts are blue, but cuprous salts are colourless. By noting, therefore, the amount of the urine (diluted, if necessary, to a known bulk) which is necessary to decolorise a given quantity of the standard Pavy's test, we obtain a measure of its reducing power, and so of the dextrose present. Again, we can adapt the fermentation test to quantitative purposes, by measuring the CO_2 produced from a definite quantity of the urine, or by ascertaining the diminution in the specific gravity of the fluid which follows the destruction of the sugar by the yeast. Lastly, we may employ the polarimeter, which indicates the percentage of dextrose by the number of degrees through which a polarised ray is turned to the right on passing through a layer of urine of determinate depth. A drawback to the use of this instrument, when applied to the urine, arises from the fact that other substances may be present which are optically active.[1]

(b) Lævulose.—The occurrence of lævulose in normal urine has not been observed; but in certain cases of glycosuria it is said to be present. Kulz[2] separated from the urine of a diabetic a lævorotatory sugar, which possessed all the properties of ordinary lævulose, except that, unlike the latter, it was precipitated by basic acetate of lead. When lævulose is given by the mouth in diabetes, it can be utilised in metabolism more readily than dextrose, and within certain limits of administration it is not excreted in the urine. Beyond these limits, however, it is eliminated partly unaltered and partly as dextrose.[3]

(c) Lactose.—That a reducing substance is apt to appear in the urine of women during the period of lactation, was first observed by Heller as far back as 1849; and F. Hofmeister,[4] in 1877, showed definitely that

[1] Details of all these processes will be found in practical handbooks.

[2] *Ztschr. f. Biol.*, München, 1890, Bd. ix. S. 228. References to the earlier literature will be found in this paper.

[3] Cf. Haycraft, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xix. S. 137; Hale White, *Guy's Hosp. Rep.*, London, 1893, p. 133.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 104.

this was milk-sugar. Only lately, however, has it been recognised that lactosuria is an almost constant phenomenon during lactation. Even when the conditions are altogether favourable and normal, it is seldom that the sugar is not present in the urine at some portion of the period. When any interruption to the natural removal of the milk occurs, the amount may be very considerable.

The phenomenon is easily understood when it is remembered that the lactose excreted into the blood from the mammary gland does not come under the normal influence of the liver. Recent researches, indeed, indicate that milk-sugar cannot in any case act as a precursor of glycogen, until it has been inverted. When lactose is taken by the mouth, this inversion occurs before or during absorption from the bowel.

The complete identification of lactose in the urine is difficult, unless it be first isolated by processes too lengthy to be described here. But if the urine exhibit the following characters, the presence of lactose is established almost without the possibility of doubt. It should reduce copper and bismuth solution; but, with the fermentation test, it should give negative results for the first twenty-four hours of the experiment, and it should give no definite crystalline precipitate with the phenylhydrazine test when this is directly applied.[1] On the other hand, after boiling with 5 per cent. sulphuric acid for a short time, the urine should, if first neutralised with ammonia, give the phenylhydrazine test readily; crystals of dextrosazone should be thus obtained, and, with proper precautions, galactosazone crystals may be also distinguished. Although the lactose is converted by the mineral acid into dextrose and galactose, a fermentation is not always to be obtained after treatment, as the large amount of sulphate, which is present after neutralising the acid, interferes with the growth of the yeast. If the reducing power of the urine be estimated, this should be found increased after boiling with mineral acid, but unaffected by boiling with citric acid.

(d) **Pentoses**—*Xylose, arabinose* ($C_5H_{10}O_5$).—Ebstein,[2] Salkowski,[3] and others have observed the presence of 5-carbon sugars in the urine. They are generally, when present, derived from the food, and then probably arise from certain fruits, especially cherries and plums, which contain either pentoses or a precursor of these sugars, the so-called "fruit gum." The pentoses are apparently assimilable with difficulty. Under exceptional circumstances, it seems possible that they may arise in the organism, as the result of disordered metabolism. It has been found that a certain proteid, derived from the pancreas, yields pentoses when boiled with acids, and some such substance may be the source of pentosuria.

The pentoses give a red coloration when treated with strong hydrochloric acid in the presence of phloroglucin (Tollens' reaction). Glycuronic acid, however, behaves similarly. They reduce copper solutions, and yield an osazone after somewhat prolonged warming with phenylhydrazine, but they do not ferment.

(e) **Isomaltose.**—When the mixture of carbohydrates obtained from normal urine by precipitation with benzoylchloride is fermented with yeast, so that all the dextrose present is destroyed, there remain small

[1] Lactosazone does not crystallise readily, except from pure solutions of the sugar.

[2] *Virchow's Archiv*, 1892, Bd. cxxix. S. 401; cxxxii. S. 368.

[3] *Centralbl. f. d. med. Wissensch.*, Berlin, 1893, S. 193; *Berl. klin. Wchnschr.*, 1895, No. 17.

quantities of a sugar, which, though not fermentable, gives a well-crystallised osazone, and reduces Fehling's solution. According to the researches of Baisch,[1] the properties of this substance agree with those of "isomaltose."

(f) Animal gum.—The third and remaining carbohydrate which separates from normal urine when this is treated with benzoylchloride, is a dextrin-like substance, in all probability identical with "animal gum." This was first isolated from urine by Landwehr,[2] who took advantage for this purpose of the insolubility of its copper compound. Its presence has been confirmed both by Wedenski and Baisch, who employed the benzoylchloride method.

The substance does not reduce metallic salts, but, on the other hand, on boiling with mineral acids, it yields a derivative which will reduce Fehling's and Nylander's reagents freely. Simultaneously it yields (like many other carbohydrates and certain of the aromatic constituents of the urine) with acids a brown flocculent precipitate of the "*humous substances*" of v. Udránsky.[3] It is due to the presence of this substance that the reducing power of most urines is increased after boiling with mineral acids.

(g) Glycuronic acid.—The chemical relationship of this acid to the glucoses is seen by a comparison of their respective formulæ:—

$CH_2HO(CH.OH)_4CHO$ (glucose) $\qquad$ $COOH.(CH.OH)_4CHO$ (glycuronic acid)

It is derived from these sugars by oxidation of the primary alcohol group, $CH_2.OH$, to the carboxyl group, COOH. It is at once, therefore, an aldehyde and an acid. As an aldehyde, it reduces copper solutions.

It should be understood that glycuronic acid is never a constituent of normal urine in appreciable amount, nor does it appear as the result of pathological processes in the ordinary sense. Its presence almost always depends upon the ingestion of special substances, which are for the most part foreign to ordinary foodstuffs; and, when excreted, it is "conjugated" with these, or with derivatives of these.

It is apparently an intermediate product in the metabolism of carbohydrates, which, normally, becomes fully oxidised in the body; but which, when conjugated with the exceptional substances referred to, escapes oxidation, and appears in the urine, just as the easily oxidisable glycin is protected by conjugation with benzoic acid and appears as hippuric acid.

Some of the substances which form these conjugated compounds with glycuronic acid, are those which ordinarily form conjugated or ethereal sulphates (cf. pp. 606 and 631), especially the aromatic hydroxy-compounds. Phenol- indoxyl- and skatoxyl-glycuronic acids and many analogues have been described in the urine. But apparently the sulphate conjugation is the more constant process, and it is only when the above substances are present in very large amount that their glycuronic conjugates appear in addition to their sulphuric acid compounds,—in general, only when they, or their precursors, are given abundantly by the mouth for the purposes of experiment.

Of more practical importance are those conjugated compounds of

[1] Baisch, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. xx. S. 249.
[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1885, S. 369.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 33.

glycuronic acid with members of the fatty group of alcohols, which are excreted after the use of certain common drugs. Thus, when chloral hydrate is being taken, trichlorethylglycuronic acid (urochloralic acid)[1] is often found in the urine, and analogous compounds arise after the administration of butylchloral hydrate and chloroform.

All these compounds are lævorotatory, though glycuronic acid itself is dextrorotatory; many of them, urochloralic acid for instance, reduce bismuth and copper solutions freely, and their presence may therefore lead to error in testing for sugar; but they are not fermentable. They split up with varying degrees of ease into glycuronic acid and the conjugated substance, either by boiling with mineral acids, or when heated with water in sealed tubes; some (*e.g.* phenolglycuronic acid) decompose when boiled with water alone.

Glycuronic acid itself is a syrupy substance, soluble in water and alcohol; but when its aqueous solutions are boiled or evaporated, it loses water, and forms a crystalline anhydride which is insoluble in alcohol.

To separate the urinary glycuronic compounds, a large quantity of urine is precipitated with acetate of lead, and the precipitate decomposed with sulphuretted hydrogen. After filtering, barium hydrate is added to the solution. The sulphates and phosphates thus precipitated are filtered off, and alcohol added to the filtrate, whereupon the barium salts of the conjugated glycuronic acids crystallise out.[2]

Other Organic Compounds.

Oxalic acid.—Small quantities of oxalic acid $(COOH)_2$ are present in all specimens of urine, about 50 mgrms. being the average for the day's excretion. Much of this may arise directly from preformed oxalates ingested with the food, as all vegetable food contains traces of these salts, and direct experiment has shown that they are susceptible of but very incomplete oxidation in the body.[3] But oxalic acid does not disappear from the urine when pure flesh-food is taken, nor even during starvation;[4] it would thus seem certain that it can arise from proteid metabolism. It is, in certain cases, very largely increased in amount, from causes which are not clearly understood. Some authorities hold that these cases of "oxaluria" depend always upon excess of preformed oxalates in the diet; but no one who has observed the marked tendency to increased oxalate excretion in diabetes, or the way in which, in some cases of glycosuria, a temporary decrease in the sugar may be associated with an increase of oxalates, can doubt that it may arise also from incomplete oxidation of carbohydrates.

Oxalate of calcium frequently separates from the urine to form a crystalline deposit. It mostly takes the form of the so-called "envelope crystals," but may appear as dumb-bells, and is often seen as clear ovoids (Fig. 56). It is responsible for the formation of a variety of urinary calculus.

[1] This was the first of these substances to be described, *vide* Musculus and v. Mering, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1875, Bd. viii. S. 662.
[2] Cf. Ashdown, *Brit. Med. Journ.*, London, 1890, vol. i. p. 169.
[3] Gaglio, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 246.
[4] Marfori, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1892, S. 72.

To *demonstrate* the presence of oxalic acid, and to *estimate* its amount, a litre of urine should be treated with calcium chloride and ammonia, and afterwards made acid with acetic acid. After twenty-four hours' standing, the crystalline precipitate, which contains uric acid crystals mixed with calcium oxalate, is filtered off and treated with dilute hydrochloric acid. The oxalate dissolves, and is reprecipitated, after filtering off the uric acid, by the addition of ammonia. At a dull red heat it is converted into calcium carbonate, and may be weighed as such.

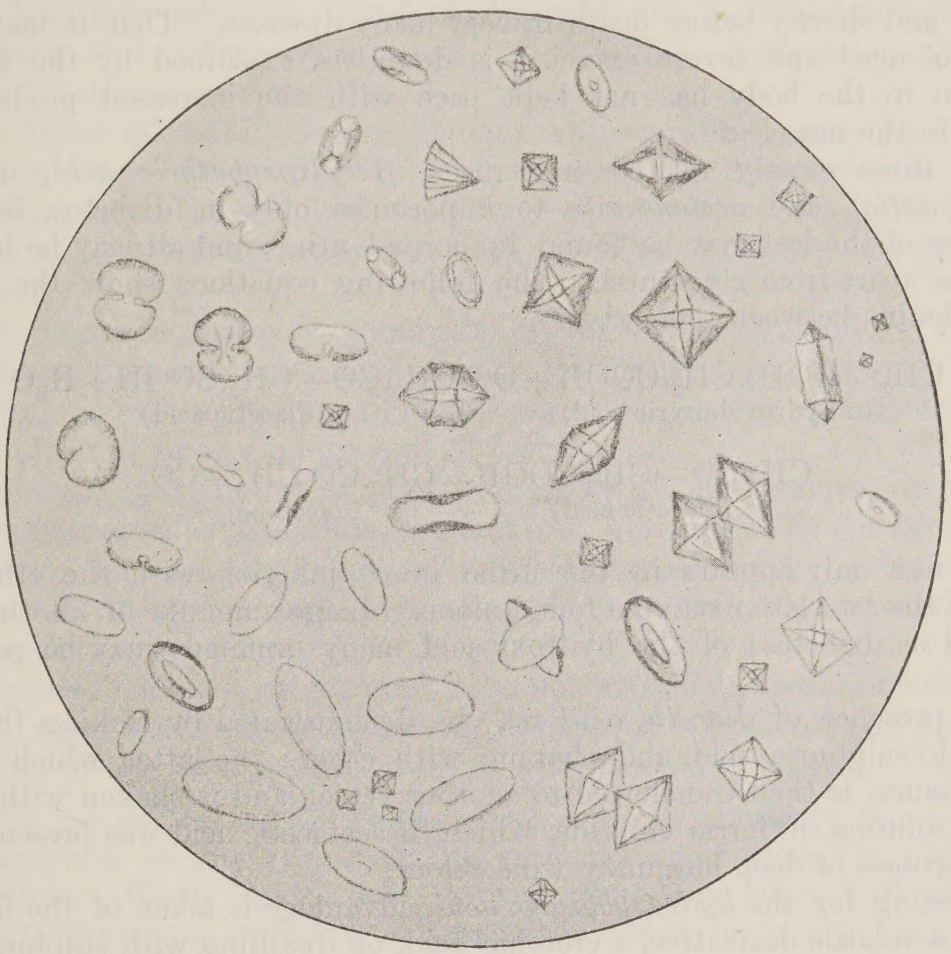


FIG. 56.—Calcium oxalate.

Acids and hydroxyacids of the fatty series, with derived substances.—Normal urine contains minute quantities of the *volatile fatty acids*, especially acetic, but also formic, propionic, and butyric acids.[1] They do not, as a rule, amount collectively to more than some 50 mgrms. in the day's excretion, and they arise doubtless from the bacterial decomposition of carbohydrates and proteids in the lower bowel. Fatty acids of low atomic weight, such as the above, are less easily oxidised in the organism than are those of greater complexity.[2]

The amount is considerable in the urine of herbivora, and in man it is increased by many diseases, especially by such as lead to increased decomposition in the bowel, or to prolonged constipation.

When a specimen of urine undergoes ammoniacal fermentation, the volatile acids are increased at the expense of the carbohydrates it contains.[3]

These acids are obtained from the urine by distillation with phosphoric

[1] Cf. v. Jaksch, *Ztschr. f. physiol. Chem.*, Strassburg, 1886, Bd. x. S. 536. The earlier literature is here summarised.

[2] C. Schotten, *ibid.*, 1883, Bd. vii. S. 375.

[3] Salkowski, *ibid.*, 1889, Bd. xiii. S. 264.

acid. They are found in the distillate so obtained, together with traces of hydrochloric and benzoic acids, phenol, and acetone.

Sarcolactic acid is not a normal component of human urine, but it appears in many diseased or abnormal conditions of the body, of which it may be said generally that they involve either a suspension of normal hepatic functions or interference with the proper oxidative processes of the body.[1] It was first observed in the urine of phosphorus poisoning, and of acute yellow hepatic atrophy,[2] and may be always demonstrated in these conditions. It is found also after slow asphyxia; in poisoning by carbon monoxide, in prolonged anæmia, and shortly before death in very many diseases. That it may appear after prolonged and severe exercise, is doubtless explained by the fact that oxidation in the body has not kept pace with the increased production of lactates in the muscles.[3]

The three closely related substances, *β-hydroxybutyric acid*, *acetacetic acid* (*diacetic*), and *acetone* rise to importance only in diabetes, but small quantities of the last may be found in normal urine, and all may be increased in disease apart from glycosuria. The following equations show the relation which obtains between them :[4]—

$$\underset{(\beta\text{-hydroxybutyric acid})}{CH_3CH(OH).CH_2.COOH} + O = \underset{(\text{diacetic acid})}{CH_3.CO—CH_2.COOH} + H_2O$$

$$\underset{(\text{diacetic acid})}{CH_3.CO—CH_2.COOH} = \underset{(\text{acetone})}{CH_3.CO.CH_3} + CO_2$$

The first only appears in the urine in conjunction with the others, but either of the two latter may be found alone. Large amounts of all three may be found in diabetes; of the hydroxy-acid many grammes may be passed in the day.

The presence of *diacetic acid* may be demonstrated by making the urine acid with sulphuric acid, and shaking with ether; the latter, which extracts the substance, is then transferred to another vessel, and is shaken with a weak aqueous solution of ferric chloride, which, if acetacetic acid was present in the urine, becomes of deep burgundy wine colour.

In testing for the *hydroxybutyric acid*, advantage is taken of the fact that it yields a volatile derivative, a crotonic acid, on distilling with sulphuric acid. This substance crystallises out from the distillate of the urine, and may be identified by its melting point (72° C.). The urinary hydroxybutyric acid is lævorotatory.

Acetone is identified in a urinary distillate by first adding a few drops of a solution of sodium nitroprusside, and then caustic alkali, whereupon, in the presence of acetone, a fine cherry-red colour is produced. Acetic acid subsequently added in excess changes the colour to purple (*Legal's test*).

The Colour of Urine and the Chemistry of the Pigments.

It is a familiar fact that, under physiological conditions, the urine may be almost colourless, or may exhibit tints varying from a pale straw yellow, through deep orange, to reddish brown. In its commonest condition it is yellow. Pathologically, the colour may undergo variations wider than those seen in health.

[1] Cf. Araki, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xix. S. 422, with reference to previous papers by this author.

[2] Schultzen u. Riess, *Chem. Centr.-Bl.*, Leipzig, 1869, S. 681.

[3] Colasanti and Moscatelli, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1890, Bd. xxvii. S. 158.

[4] Cf. Minkowski, *ibid.*, 1893, Bd. xxxi. S. 183.

An effort has been made to refer the varying degree of pigmentation to a standard colour scale, so that the condition of a given specimen, as regards colour, might be quantitatively expressed. But much difficulty intervenes, in that variations may be due, not alone to differing amounts of a single colouring matter, but to independent and quite irregular variations in at least three or four. The endeavour to attain to quantitative precision has on this account proved unsuccessful in practice.[1] We may content ourselves with speaking of physiological urine as pale, normal, or high-coloured respectively, and assist the description by comparison with other substances of familiar appearance ("straw-coloured," "sherry-coloured," etc.).

Pale urine is usually of low density, and contains a small proportion of solid matter. It results from all causes which promote a copious flow of fluid from the kidneys, such as free ingestion of liquids, a check to the cutaneous transpiration (as from the effect of cold), and emotional excitement.

High-coloured urine is generally of high density, and is excreted when the transpiration from the skin is more than usually free, or under conditions of high metabolic activity. After a full meal the urine is often at once copious and of full colour.

In general the amount of pigment rises with an increase in the constituents excreted by the renal epithelium, and not with the glomerular excretives. The depth of colour may be affected by the reaction of the urine; other things being equal, an acid urine will show a darker tint than one that is alkaline.

Examined directly by means of the spectroscope, fresh normal urine is found nearly always to show no definite absorption-band; a diffuse absorption of the more refrangible rays being alone evident.

But by the aid of the *spectrophotometer*[2] we may measure the amount of light absorption in any region of the spectrum apart from the presence of actual bands. When light passes through urine, the amount of absorption increases progressively from the mid-red to the violet.

Suppose the absolute absorption at any two points in this region of spectrum be measured; say in the neighbourhood of the Fraunhofer lines, E and F respectively. If in any one specimen of normal urine the absorption near F is found to be twice as great as that near E, then if the urine contained but one pigment, this same ratio would be found in any other specimen. The absorption at F would in all cases be double that at E. For, clearly, the dilution of an individual pigment would decrease the *absolute* absorption throughout the spectrum, but would leave the *relative* absorption at any two points unaffected; similarly, concentration would increase the absolute, but would nowhere affect the relative, absorption. But different specimens of normal urine do not agree in this way. One urine may show more relative absorption (say) in the mid-green, another more in the blue. This can only be due to the fact that more than one pigment is concerned.[3] Although it yields no definite bands, the spectroscopic properties of fresh normal yellow urine thus indicate some complexity in its pigmentation; but the same experimental evidence indicates nevertheless that no more than one pigment is usually present in a relatively large amount.

[1] By the use, however, of Lovibond's tintometer, the colour of urine under varying circumstances may be very exactly imitated, and expressed in terms of a scale.

[2] See this textbook, article "Hæmoglobin," p. 213.

[3] This argument only holds for colouring matters which do not undergo dissociation in solution.

The pigments of the urine have long received attention and have been the subject of many laborious researches; but, owing to the great difficulties they present to the investigator, our knowledge of the chemistry of most of them has remained indefinite. These difficulties arise from various causes. Pigment metabolism appears to be always of a highly conservative nature. The colouring matters found in the epidermal structures of animals, serving for ornament, protection, or other purposes, are almost always present in strikingly small quantity; and those which are purely excretory leave the body in equally small proportionate amount.

The highly developed optical activity of these substances, which has led us to group them together in a special class as "pigments," at the same time gives to them a prominence in various phenomena, disproportionate to the actual quantity in which they are present. The urinary pigments are (at least, under normal conditions) quite minute in amount, and this fact is the primary difficulty in the path of chemical investigation. As Bunge has written, many endeavours have resulted merely in applying Greek and Latin names to substances which have been obtained in quantity too small for proper investigation.

The extremely delicate indications of the spectroscope have been of the greatest assistance in overcoming this fundamental difficulty, and our knowledge of pigments has been much extended by its use. But evidence so gained has to be checked and assisted by other methods. A complex spectrum may indicate a mixture of substances; but it may, with equal probability, be due to one alone. A mixture, on the contrary, may show but a single absorption-band, for the reason that of the pigments present one alone extinguishes light in a specific region.

It is therefore easy, by a mere qualitative use of the spectroscope, to mistake a mixture for a chemical individual. On the other hand, very slight variations in the physical condition of a pigment, or a minute change in its molecular constitution, may produce a great effect upon its spectrum, and, unless we are aware of these conditions, we may be led to see wide differences where chemically there is little or none.

When, again, endeavours are made to isolate pigments by chemical means, the great instability which they exhibit as a class is apt to lead to error. So often has this danger been overlooked, that we are compelled to attach no importance, beyond what accrues from historic interest, to much of the work which has been done on this problem.

It is of prime importance, when we endeavour to obtain these unstable substances in their integrity, that the use of highly active reagents should be avoided.

We shall deal only with the pigments of which we have comparatively accurate knowledge; but it may be safely asserted that the four substances now to be described form the basis of urinary chromatology. These are *urochrome*, *urobilin*, *uroerythrin*, and *hæmatoporphyrin*. Other pigments exist, and some have doubtless yet to be recognised, but they are exceptional, or take but very small share in the coloration of the urine.

Preformed pigments of normal urine—(a) The essential yellow pigment, urochrome.—In 1864, Thudichum gave the name of urochrome to preparations obtained from normal urine by complicated processes of extraction. Thudichum's products undoubtedly contained a large pro-

portion of the substance we are now to describe, but they were mixed with urobilin and with decomposition products.

To A. E. Garrod[1] we owe a process for the extraction of the essential yellow colouring matter, which is beyond reproach in its avoidance of destructive reagents. It yields a product entitled to be considered, with a large degree of probability, as a chemical individual.

Following Garrod, we shall describe this pigment under the name of *urochrome*—a name eminently fitted for a substance which is the prime cause of the familiar colour of urine, but in the use of which we must avoid historical confusion.

Between the urochrome of Garrod and that described thirty years earlier there is the difference between presumptive chemical individuality and almost certain admixture. It should be stated, however, that Thudichum still holds the pigment described by him to be a definite substance, and has recently investigated certain of its reactions, which he believes indicate for it the combined characters of an alcohol and a base.

Separation of urochrome (Garrod).—The urine is saturated with crystals of ammonium sulphate, and, after standing, is filtered (*vide infra*, "Separation of Urobilin"). The filtrate, which is still almost as highly coloured as the original urine, is shaken with alcohol. The latter solvent separates rapidly from the saline mixture, and is seen to withdraw a large proportion of the colouring matter. Repeated extraction removes practically all. The alcoholic solution is diluted with a large bulk of water, and the mixture again saturated with ammonium sulphate; by this procedure the alcohol is again made to separate from the water, carrying the pigment with it, and a satisfactory washing of the original extract is secured. This second alcoholic solution is now made just alkaline with ammonia, and evaporated to dryness; the residue is extracted once or twice with acetic ether, which removes certain impurities, and is again dissolved in strong alcohol. Somewhat prolonged digestion is necessary at this stage, as the solubility in alcohol is decreased when the pigment has once been taken to dryness. Finally, the alcohol is concentrated till it has a deep orange colour, and is poured into at least an equal volume of ether, when an amorphous brown precipitate falls, consisting of the greater portion of the pigment present, in almost pure condition. The precipitate may be filtered off, dried on the paper, and washed with a little chloroform and absolute alcohol.[2]

Properties.—The substance so prepared is a hygroscopic, brown, amorphous substance, easily soluble in water, much less soluble in alcohol; only slightly soluble in acetic ether, amyl alcohol, or acetone; and insoluble in chloroform, ether, and benzene. Its solutions show no definite spectroscopic absorption-bands, even after the addition of acids. Zinc chloride and ammonia produce no fluorescence. Alkalies give the solution a brownish tint, acids a reddish brown. The pigment forms insoluble compounds with the heavy metals, and is precipitated by phosphotungstic and phosphomolybdic acids. With strong nitric acid it undergoes a colour change resembling the xantho-proteic reaction.

Urochrome we have seen to be a pigment which can be removed from the urine without the use of strong reagents, and in the removal of which the fluid loses nearly all its colour. At the same time, its aqueous

[1] *Proc. Roy. Soc. London*, 1894, vol. lv. p. 394.

[2] See also Kramm, *Deutsche med. Wchnschr.*, Leipzig, 1896, Bd. xxii. S. 25 and 42. This author separates the pigment by an entirely different method. He confirms Garrod's original account of its properties.

solutions have a tint like that of urine itself, and, like normal urine, show no absorption-bands. There can be no doubt, therefore, that it is the essential cause of normal urinary coloration.

Physiological relations.—Until quite recently, we had no knowledge of the chemical relationship, or of the metabolic precursors of this important physiological pigment. But Riva [1] and Chiodera [2] have obtained, by the action of potassium permanganate upon solutions of urobilin, a substance which they believe to be identical with urochrome. A. E. Garrod [3] has added still more conclusive evidence for the existence of a simple relation between this pigment and urobilin, by observing that alcoholic solutions of pure urochrome, when treated with aldehyde (which we may believe acts as a mild reducing agent), yields a pigment showing the spectrum and all the more characteristic properties of urobilin. The establishment of this relation is most important in bringing our knowledge of physiological pigments into line, since, as will be shown immediately, the derivation of urobilin from blood and bile pigments is clearly established. We can now ascribe a similar origin to the fundamental colouring matter of urine.

(b) **Urobilin.**—In 1868, Jaffé,[4] as an outcome of a spectroscopic study of the urine, discovered a pigment with well-characterised properties, to which he gave the name of urobilin.

This pigment is perhaps scarcely entitled to be classified among the preformed pigments of normal urine, for it is present as a rule in minimal amount and almost always in the form of a chromogen. But on rare occasions the free pigment is found in the fresh urine of normal individuals, and, moreover, the importance of urobilin in other respects makes it necessary to give it a prominent place in this section. It was the first physiological urinary pigment of which we had accurate knowledge from the point of view of genesis and metabolic history. Its increase in disease is a familiar phenomenon. These facts and its well-marked spectroscopic characters have made it predominant in the literature of urinary pigments. Even at the present time it is sometimes described as the essential colouring matter of urine, an error which is at once demonstrated if the spectroscopic indications of normal urine and of weak solutions of pure urobilin are compared.

Separation.—When zinc chloride and ammonia are added to urine in due proportion, a precipitate is obtained (cf. "Separation of Creatinin," p. 599), which contains much of the urobilin present. This method of precipitation was used by Jaffé, and from the zinc precipitate he succeeded in extracting the pigment in a remarkably pure condition, but in small quantity, and by a somewhat complicated procedure.

Méhu [5] later showed that saturation of the urine with ammonium sulphate, after acidification with weak sulphuric acid, produced a complete precipitation of this pigment. The precipitate thus produced, which mainly consists of pigmented urates, will yield to acid alcohol a solution, in which the characteristic absorption-band of acid urobilin, to be later described, is easily seen. Even when normal urine has been employed, the spectrum may be observed after this procedure, for the bandless chromogen is decomposed by the acid

[1] *Gazz. med. di Torino*, 1896, vol. xlvii. No. 12.
[2] *Arch. ital. di clin. med.*, Milan, 1896, vol. xxxv. p. 505.
[3] *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 190.
[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1868, Bd. vi. S. 243 ; *Virchow's Archiv*, 1869, Bd. xlvii. S. 405.
[5] *Bull. Acad. de méd.*, Paris, 1878, tome vii. p. 671.

employed. When a urine rich in urobilin is saturated with ammonium sulphate (best after previous removal of the urates by preliminary saturation with chloride of ammonium), and acidified with sulphuric acid, it will yield the pigment when shaken with a mixture of ether and chloroform. From this organic solvent distilled water will again remove all the urobilin, and from the water it may be precipitated by the further use of ammonium sulphate. A method of separation may be based upon these facts which will yield a very pure product in comparatively large amount.[1]

Properties.—Urobilin is an extremely soluble substance, dissolving freely in all ordinary solvents. It is, however, proportionately less soluble in water than is urochrome, though much more readily soluble than the latter in alcohol and other organic liquids. Its solutions, when concentrated, have a brown colour; when more dilute they are yellow; on great dilution they exhibit a highly characteristic change to a dull pink colour.

An alcoholic solution of the pure pigment free from extraneous acid or alkali exhibits a green fluorescence quite apart from the addition of reagents. When, however, zinc chloride and ammonia are added, a greatly increased fluorescence is produced. This striking reaction is of much value in the identification of urobilin; it may be obtained after great dilution.

Solutions of urobilin exhibit very definite *spectroscopic phenomena.* In clear acid solutions of moderate strength, a single absorption-band is seen between the Fraunhofer lines *b* and F, slightly overlapping the latter; situate, therefore, at the junction of the green and blue of the spectrum (Fig. 57, Spectrum 4). In highly concentrated solution this band is lost in a general absorption of the more refrangible rays. On diluting such a concentrated solution a broad band first appears with a region of complete blackness towards red, and a dark shading towards violet. As dilution proceeds the shading first disappears, and then the dark portion of the band shrinks till its limits extend from about λ 508 to λ 486. After this the width of the band is constant, until with very large dilution it grows faint and ultimately disappears (Fig. 57, Spectrum 5). The activity of the pigment in absorbing light in this region is enormous, and a solution so dilute as to have a very faint colour indeed, will show a well-marked band. An absorption-band of an intensity such as is occasionally seen in normal urine, would correspond to that of an almost colourless solution of the pure substance.

Urobilin, like most animal pigments, shows acidic tendencies, and forms compounds with bases, being liberated from these combinations on the addition of an acid.

If ammonia be added to a solution of the free pigment, the colour changes to a canary-yellow, and unless the solution be very strong the absorption-band disappears. The sodium and potassium compounds have a colour in solution more like that of the free pigment, and show an analogous band, which is situate, however, somewhat nearer the red. The zinc compound in ammoniacal solution fluoresces, as we have already stated, and shows with the spectroscope a band almost identical with that of the potassium and sodium compounds. The calcium compound is yellow in solution and shows no band. Mercury forms a pink compound, with a band nearer to the red than any of those previously referred to. A solution of mercuric chloride will develop a pink colour

[1] Garrod and Hopkins, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 120.

when applied to tissues stained with urobilin, and may thus be used as a test for such staining (Adolf Schmidt).

When to a concentrated solution of nearly pure urobilin in sodic or potassic hydrate, sufficient sulphuric or hydrochloric acid is added to render the liquid faintly acid, a slight turbidity is observed, due to the liberation of the free pigment from its more soluble alkaline combination. If the turbid liquid be examined with the spectroscope, there is seen, in addition to the ordinary acid band between *b* and F, a sharply-defined narrow band in the green, enclosing, and being almost bisected by the Fraunhofer line E (Fig. 57, Spectrum 6). This extra band is most probably due to the special light absorption exercised by the impalpable particles of solid urobilin in suspension. It wholly disappears when the precipitate is filtered off, or when it is redissolved, the ordinary band alone being then visible.[1]

Solid urobilin is an amorphous red-brown substance, which, when isolated and dry, may be kept without decomposition. It is not deliquescent, but fuses at comparatively low temperatures, afterwards solidifying to a brittle transparent shellac-like form. It has a slight but peculiar and characteristic odour.

Physiological relations.—Urinary urobilin is identical with the chief pigment of fæces (stercobilin). So certain is the identity of these two substances, that it is undesirable to retain separate names for them.

Urobilin is closely related to the pigments of the bile. This was from the first recognised by Jaffé; and shortly after the discovery of the pigment, Maly prepared a substance (hydrobilirubin) by the reduction of bilirubin with sodium amalgam, which he held to be identical with urobilin. That the urinary pigment is a reduction product of bilirubin is likely, but it is probable that hydrobilirubin, as described by Maly, represents an intermediate stage in the reduction. It differs at any rate somewhat from urobilin as it occurs naturally.

Urobilin is formed, however, when bile decomposes out of contact with the air, and it may be extracted from the bile removed post-mortem from the gall bladder.

Several observers have shown that intestinal micro-organisms can effect the reduction of bilirubin to urobilin.

This pigment, or substances closely allied to it, can be prepared direct from hæmoglobin derivatives—hæmatin and hæmatoporphyrin—by reduction processes. It has been stated that oxidation is also capable of yielding urobilin from bile and blood pigments respectively, but it is not conceivable that both reduction and oxidation could lead to the same chemical result, and there is in this matter an anomaly which requires explanation. It must not be forgotten that peroxides (peroxide of hydrogen and peroxide of lead, have been employed in this connection) may in a sense act as reducing agents, free oxygen being given off by the interaction of the peroxide and any easily reducible compound with which it is brought in contact.

Urinary urobilin has not yet been analysed. If the formula of hydrobilirubin be compared with those of the related pigments, it will be seen that both reduction and hydration probably occur in its formation.

Hæmatin . . .	$C_{32}H_{32}N_4O_4Fe$
Bilirubin . . .	$C_{32}H_{36}N_4O_6$
Hydrobilirubin . .	$C_{32}H_{40}N_4O_7$

[1] Garrod and Hopkins, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 125.

The change from bilirubin to hydrobilirubin may be thus expressed—

$$C_{32}H_{36}N_4O_6 + H_2O + H_2 = C_{32}H_{40}N_4O_7$$

If urobilin differs from hydrobilirubin, the difference is possibly, as already stated, in the direction of increased reduction.

The *origin* of urinary urobilin is probably threefold—from absorption of the ready-formed pigment in the bowel; from direct production in the liver; and, lastly, from reduction of the blood pigment in the organs, independently of hepatic agency.

Of the precise nature of the *chromogen* of urobilin we have no knowledge. It is precipitated intact when normal urine is saturated with ammonium sulphate in the absence of mineral acid.[1] It is possible that oxidation may decompose it, as some urines originally showing no absorption-band will develop such on standing. This phenomenon might follow, however, from the decomposition during standing of some compound of the pigment with lime or other base.

(c) **Uroerythrin.**—This pigment is best known as the colouring matter of pink urate deposits. It is a substance of the greatest interest, but one which has proved, from its marked instability, elusive and difficult of investigation.

It was first dealt with as far back as 1800, by Louis Proust, under the name of *acide rosacique.* Its present name was assigned to it by F. Simon in 1850—the term "purpurin," earlier proposed by Golding Bird, being still sometimes used. Heller published an account of the pigment in 1854, and Macmunn first accurately described its spectrum in 1883. Very important contributions to our knowledge of uroerythrin have recently been made by Riva, Zoja, and A. E. Garrod.[2]

The quantity of the pigment excreted is, under any circumstances, very small; but its tinctorial power is extremely high, and when in solution it may materially contribute to the coloration of the urine. It is certainly to be looked upon as a pigment of normal urine, as urates coloured by it frequently separate from the excretion of persons in health.

Separation.—A quantity of pink urate deposit is collected upon a filter, washed with ice-cold water, dried, and soaked in absolute alcohol. The alcohol, though a solvent for uroerythrin, does not extract it from the urates. The spirit is poured off and the precipitate dissolved in warm water; from the aqueous solution so obtained the pigment is easily and completely extracted by shaking with amylic alcohol (Riva). Garrod has shown that if the pink urates are first dissolved in warm water, and are then reprecipitated by saturation with ammonium chloride, the pigment is carried down with them afresh, and in such a condition that it may now be extracted with alcohol. An alcoholic solution, if diluted with water, may be washed by shaking with neutral chloroform, which removes impurities but no uroerythrin. But if after this preliminary washing a fresh supply of chloroform is added, together with a single drop of acetic acid, on shaking, the pigment is now found to be transferred completely to the chloroform as an effect of the acidification of the liquid.

Properties.—The most striking properties of uroerythrin are—(1) Its remarkable affinity for uric acid compounds; (2) the ease with which

[1] Eicholz, *Journ. Physiol.*, Cambridge and London, vol. xiv. p. 326.

[2] *Journ. Physiol.*, Cambridge and London, 1895, vol. xvii. p. 439. Full references to the literature will here be found.

its solutions are decolorised by light; and (3) its colour reactions with the caustic alkalies and mineral acids. The pigment invariably associates itself with urates during their precipitation; either when they separate naturally from a urine containing it, or when they are artificially added to its pure solutions, and are allowed afterwards to separate.

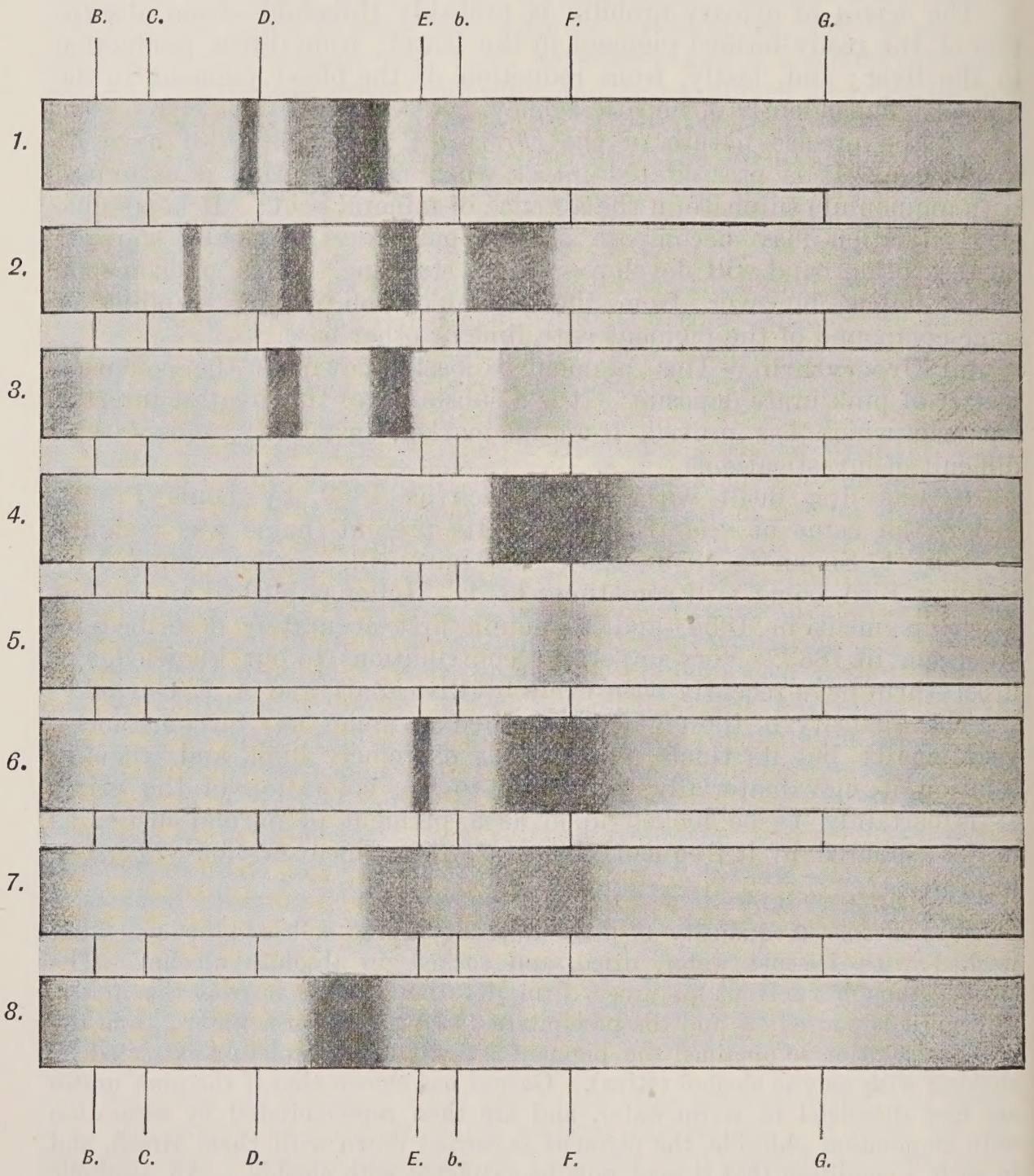


FIG. 57.—Chart of spectra.

1. Acid hæmatoporphyrin.
2. Alkaline hæmatoporphyrin.
3. Hæmatoporphyrin as found in urate sediments.
4. Acid urobilin—concentrated.
5. Acid urobilin—dilute.
6. The E band spectrum.
7. Uroerythrin.
8. Urorosein concentrated—on dilution the band shrank rapidly from redward end.

It apparently forms a loose compound with the urates, as a special absorption-spectrum is seen when light passes through the pink precipitate, differing from that proper to solutions of the pigment (Garrod).

The best solvent of uroerythrin is amylic alcohol; acetic ether is but little inferior, and the pigment is also soluble in alcohol, chloroform, and

water. The solutions have a rich orange colour; only when very dilute and quite free from impurity do they exhibit a pink tint. All solutions of the pigment are decolorised on exposure to light, even to subdued daylight. On the other hand, light has little effect upon the solid pigment, and none at all upon pink urate sediments.

When solid uroerythrin is treated with solutions of the caustic alkalies, a remarkable green coloration is produced (Thudichum). Green derivatives from animal pigments are so uncommon that the reaction is highly characteristic. It can be well seen when a little pink urate deposit is collected upon a filter, dried, and then touched with a drop of sodium-hydrate solution. If a solution of the pigment be treated with the same reagent, a rapid play of colours may frequently be seen, from pink, through purple and blue, to grass-green. With acids, colour-reactions also occur, but they are somewhat less certain, being dependent upon exact conditions of experiment. If to a solution of the pigment sulphuric acid be added, the deep orange colour changes to a brilliant carmine. Hydrochloric acid produces a rose-pink, phosphoric acid a salmon-pink.

Examined spectroscopically, a solution of uroerythrin, at a suitable degree of dilution, shows two somewhat ill-defined absorption-bands united by a shading of less intensity (Macmunn). The more red-ward of these is seen in the green between the lines D and E, and nearer the latter; the other closely agrees in position with the ordinary urobilin band at F (Fig. 57, Spectum 7). Pink urate sediments and the carmine derivative produced by sulphuric acid agree in giving a single banded spectrum, namely, a broad band extending from the D line towards violet.

(d) **Hæmatoporphyrin.**—In 1881, Neusser[1] and Macmunn[2] observed the occurrence in urine of pigments closely related to hæmatoporphyrin. During the following decade the work of le Nobel, Stockvis, Salkowski, Hammarsten, Copeman, and others extended this discovery, and it became established that hæmatoporphyrin itself is a constituent of certain pathological urines. In 1892, A. E. Garrod[3] showed that it is also to be found in normal urine.

In health the pigment is excreted in very small amount, and can scarcely be said to function as an active colouring matter of the urine; but it is of the highest interest to recognise that this iron-free derivative of hæmatin, which in the laboratory is only to be obtained by the use of strong reagents, is a normal physiological product. In pathological conditions, and especially after the use of certain drugs, it is present in greatly increased amount.

Isolation from normal urine.—The method recommended by Garrod depends upon the fact that the pigment is carried down by the precipitate of phosphates produced on the addition of caustic alkali to the urine. After special treatment of this precipitate, the pigment may be obtained in chloroform solution. The chloroform is evaporated, and the residue washed with neutral alcohol and dissolved in acidified alcohol, when a solution is obtained of pure pink colour, comparable with solutions of the purest specimens of the pigment obtained from blood, and showing the spectrum of acid hæmatoporphyrin with distinctness.

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1881, Bd. lxxxiv. S. 536.
[2] *Proc. Roy. Soc. London*, vol. xxxi. p. 206.
[3] *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 598; *ibid.*, 1894, vol. xvii. p. 349.

Pathological urines rich in the pigment will generally yield it easily to acetic ether and to amylic alcohol.

Properties.—An account of the properties of hæmatoporphyrin will be found in the section devoted to blood pigments; but the pigment as found in the urine has certain peculiarities which must be referred to here.

When the urine is sufficiently rich in the pigment for the absorption-bands to be visible without treatment (always a pathological condition), it is found that the bands observed are those of the so-called alkaline hæmatoporphyrin (Fig. 57, Spectrum 2). Indeed, if a solution of the pigment showing the acid spectrum (but, of course, free from excess of mineral acid) be added to urine, the bands are seen to change to those of the alkaline form, even though the urine itself be of normal acidity. Acid sodium phosphate will, in fact, yield base to the hæmatoporphyrin, unless, indeed, the salt is in great excess, when it can, on the other hand, convert the alkaline form of the pigment into the acid. These facts form an interesting commentary on what we have said in the section devoted to the acidity of the urine, as to the complex conditions which govern the phenomena of chemical reaction in the fluid.

Urinary hæmatoporphyrin may be in the form of unstable modifications. Alkaline solutions of the pigment obtained from many specimens exhibit a five-banded instead of a four-banded spectrum (Macmunn). Occasionally, too, urate sediments may be pigmented with a form of the pigment which, in alkaline or neutral solution, shows a spectrum of two bands resembling that of oxyhæmoglobin (Fig. 57, Spectrum 3). Dilute mineral acids, however, promptly change this spectrum to that of ordinary acid hæmatoporphyrin (Fig. 57, Spectrum 1). There is some evidence that a colourless chromogenic substance, related to hæmatoporphyrin, may occur in the urine, as the pigment has been observed to increase in amount after standing.

Chromogenic substances in urine.—Two, at least, of the pigments we have now described (urobilin and hæmatoporphyrin) may exist, as we have seen, in the form of chromogens—colourless, or less coloured, precursors. But the urine contains other chromogenic substances, which in the original urine always, or nearly always, retain their colourless form; and, as a rule, take no share in the true pigmentation of the fluid.

We do not include, under the term of "chromogen," all substances which, by the action of strong reagents, happen to be capable of yielding a coloured derivative.

We purposely exclude such bodies as the so-called "humous substances" of Udránsky—indefinite products of wholly doubtful nature—obtained by such processes as fusing urinary precipitates with caustic alkali, or boiling the previously concentrated urine for hours with hydrochloric acid. These are probably derived from the carbohydrates and other constituents of the urine, by the destructive action of the reagents. Beyond the fact that they happen to be amorphous, and yellow or brown in colour, there is nothing to suggest that they are related to urochrome or any other definite pigment.

We shall deal only with those chromogenic substances which are of importance, either because they may, though with great rarity, appear as actual pigments, or because they yield their coloured derivatives with comparative ease, and may thus lead to confusion when the urine is being investigated in other connections.

(a) Indoxyl (indigo-blue and indigo-red).—Indoxyl (cf. pp. 607 and 631) easily oxidises to indigo-blue, or to the isomeric substance indigo-red. The relation between indoxyl and its blue derivative is expressed by the following equation:—

$$C_6H_4\begin{cases}C(OH)\\HN\end{cases}\!\!>CH + O_2 = C_6H_4\begin{cases}CO\\NH\end{cases}\!\!>C=C\begin{cases}CO\\NH\end{cases}\!\!>C_6H_4 + 2H_2O$$

(indoxyl) (indigo-blue)

The formula of indigo-red is $C_6H_4\begin{cases}CO\\NH\end{cases}\!\!>C=C\begin{cases}C(OH)\\C_6H_4\end{cases}\!\!>N$, and it arises, like its blue isomer, when, by oxidation, four atoms of hydrogen are removed from two molecules of indoxyl. Oxidising reagents when added to urine may, according to the conditions of the experiment, give rise to the formation of either or both of these coloured derivatives. The blue substance, however, is more easily and more generally obtained.

It is of great rarity for the urine to be actually pigmented with indigo-blue. As we have already seen (p. 607), the urinary indoxyl is excreted in the form of a conjugated sulphate, and this compound resists oxidation. Only when the indoxyl is first liberated from its combination does the action of oxidising reagents produce the blue colour. It is stated, however, that the urine of cholera may sometimes exhibit a blue shade from the presence of indigo-blue. We have seen that the amount of indoxyl sulphate is increased in the urine whenever bacterial putrefaction of albuminous substances is occurring to a greater extent than usual, whether in the bowel or elsewhere in the body (putrid abscesses, etc.). The most ready method of demonstrating the amount of indoxyl is by converting it into indigo-blue. *Jaffé's test.*[1]—The urine is mixed with an equal bulk of strong hydrochloric acid, by which means the "indican" (indoxyl sulphate) is decomposed and the indoxyl liberated. With a pipette, a solution of a hypochlorite is now added to the mixture drop by drop, when, by oxidation of the indoxyl, indigo-blue is formed. By shaking up the liquid with chloroform, a solution of the blue substance is obtained in the latter (Stockvis, Senator, and others). Otherwise, a crystal of potassium chlorate is placed at the bottom of a test tube and covered with the urine to be examined. Strong hydrochloric acid is then allowed to run down the side of the tube so as to reach the crystal without mixing with the urine. The latter floats upon the acid, and at the junction of the fluids a blue ring is seen of intensity varying with the amount of indoxyl present.

But indigo-blue is itself an easily oxidisable substance. It is instantly decolorised by nitric acid, and without difficulty by hypochlorites. In Jaffé's test, as above described, it is therefore necessary to add the oxidising agent with great care, or the blue colour will disappear as soon as formed. In Obermayer's[2] method, the urine is first precipitated by acetate of lead, and filtered; to the filtrate is added an equal bulk of strong hydrochloric acid, containing two or three parts per thousand of ferric chloride. The mixture is shaken for a short time, and the liberated pigment taken up, as before, in chloroform. In

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1870, Bd. iii. S. 448.
[2] *Wien. klin. Wchnschr.*, 1890, S. 176.

this case the ferric salt acts as a mild oxidising agent, sufficient to form but not to destroy the pigment.

With care a certain amount of indigo-blue may be obtained from most normal urines; and, apart from the increase in actual disease, indoxyl may be present in considerable amount, and the urine yield a well-marked indigo reaction, when nothing more than constipation exists.[1]

Indigo-red is more liable to be formed from the urinary indoxyl when Jaffé's test is applied with the aid of gentle heat. Higher temperatures favour the formation of the red isomer, lower temperatures the blue.[2] In Weber's test for indicanuria both pigments are formed. The urine is treated, as in other methods, with its own volume of hydrochloric acid; one to three drops of dilute nitric acid are then added, and the mixture heated to boiling. After cooling it is shaken with ether, when the urine, if rich in indoxyl, is found to retain a blue colour, while the supernatant ether is red or violet. The formation of indigo-red has no significance beyond such as is attached to that of indigo-blue. It may sometimes arise from the urine on the addition of strong hydrochloric acid alone (*infra*).

(b) **Urorosein.**—Quite distinct from indigo-red is the red pigment, named "urorosein" by Nencki and Sieber,[3] and since carefully studied by H. Rosin.[4] It is produced from its chromogen by the action of mineral acids; best with the aid of an oxidising reagent, but frequently appearing when the urine is treated with strong hydrochloric acid alone, especially after standing. It is freely taken up, after its formation, by amyl alcohol, but is not soluble in ether. Alkalies immediately destroy its colour. The chromogen of urorosein is precipitated by saturation with ammonium sulphate.[5]

(c) **Skatoxyl-red,** which is formed from skatoxyl on oxidation, is never obtained from urine under ordinary circumstances (Rosin), though it may be produced in the urine of animals when skatoxyl has been given by the mouth (Brieger).

It may be stated generally that when a red colour is produced in urine by the addition of strong acids (with or without the assistance of oxidising reagents), it will in the great majority of cases be due to urorosein or to indigo-red. The two pigments may be easily distinguished, in that urorosein, unlike the indigo pigment, is not taken up on shaking with ether or chloroform, and is easily decolorised by alkalies.[6]

The Pigmentation of Pathological Urines.

All the pigments and chromogens that we have so far described may be excreted in increased amount in disease. There are other pigments which only appear in the urine pathologically.

In the urine of fever a well-marked band of urobilin may generally be seen without preliminary treatment, and uroerythrin is often present in more

[1] Cf. v. Jaksch, "Klinische Diagnostik," 1896, Aufl. 4, S. 406.
[2] Rosin, *Virchow's Archiv*, 1891, Bd. cxxiii. S. 519.
[3] *Journ. f. prakt. Chem.*, Leipzig, 1882, Bd. xxvi. S. 333.
[4] *Deutsche med. Wchnschr.*, Leipzig, 1893, S. 51.
[5] Garrod and Hopkins, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 134.
[6] Rosin, *Virchow's Archiv*, 1891, Bd. cxxiii. S. 519.

than normal amount. I have also frequently observed that urochrome itself takes a share in the increased pigmentation of febrile urine.

Urobilin is found in large amount when extensive hæmolysis, or large internal hæmorrhages, have occurred; it is also greatly increased in certain cases of hepatic cirrhosis. The high colour of the urine of pernicious anæmia is in part due to urobilin; other pigments may take a large share in the increase in colour, but it is characteristic of this disease for free urobilin to be present instead of the chromogen, for even in pale specimens, which are sometimes passed, an absorption-band between *b* and F is usually visible. It is common for free urobilin to be present in diabetes. An increase of uroerythrin is seen in many forms of hepatic disorder. Hæmatoporphyrin does not appear to depend upon hæmolysis for increased excretion. After excessive use of drugs of the sulphonal type, the urine may exhibit a deep port-wine colour; part, but not the whole, of this pigmentation is due to an enormously increased excretion of hæmatoporphyrin, which may be quite unassociated with any decrease in the hæmoglobin of the blood. Increase of this pigment, but of much slighter degree, occurs also in plumbism and in certain other diseased conditions.

The so-called pathological urobilin.—Several observers have made a distinction between normal urobilin and a pathological form of the pigment. The differences found have been mainly those of spectroscopic appearances; the pathological form showing a proportionately broader band between *b* and F, and additional bands elsewhere. The various descriptions of the pathological pigment are in no sense consistent one with the other. Evidence has recently been brought forward to show that the points of distinction may be all explained as the result of impurities, and that urobilin is one and the same substance wherever found.[1]

Special pathological pigments—*Blood pigments.*—In *hæmaturia*, due to whatever cause, the urine usually contains unaltered hæmoglobin. In general the pigment may be recognised in solution spectroscopically, while red blood corpuscles are found in the deposit. Some specimens of urine preserve the integrity of the corpuscles very completely, and in slight cases of hæmaturia, while no pigment may be found in solution, the deposit obtained by centrifuging will show a red layer of corpuscles. In *hæmoglobinuria* the pigment is passed wholly in solution, and no corpuscles are found. Not infrequently *methæmoglobin* is present in place of or in addition to oxyhæmoglobin, even when the urine is first passed. Specimens which are spoken of as "smoky" usually contain this latter form of pigment.

If the quantity of pigment is too small to show a recognisable spectrum direct, the urine may be heated with caustic alkali, filtered, and a few drops of ammonium sulphide added. The more powerful absorption-bands of hæmochromogen will then be generally visible. Or, the urine may be boiled with caustic alkali, when, in the presence of blood, a greenish tint is produced, and the phosphates are precipitated with a brownish-red colour, due to hæmatin (Heller). The blue colour produced by the addition of guaiacum tincture and an ethereal solution of hydrogen peroxide, is a delicate but not wholly conclusive test when applied to urine.

Bile pigments appear in the urine in most cases of jaundice, generally in the form of bilirubin, when the urine is saffron-coloured; but occasionally partly as biliverdin, when a greenish tint predominates. When present in large amount, there is no difficulty in the recognition of these pigments. Gmelin's reaction is obtained by allowing the urine to run gently on to the surface of some fuming nitric acid contained in a test tube. The test is made more delicate if the urine be first repeatedly filtered through a clean white

[1] Hopkins, *Guy's Hosp. Rep.*, London, 1893, vol. l. p. 363; Garrod and Hopkins, "The unity of Urobilin," *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 130.

filter paper; the paper is stained yellow, and a drop of fuming nitric acid allowed to fall upon it produces the characteristic play of colours. When only traces of the pigment are present, Gmelin's test is best applied to the precipitate produced in the urine by the addition of lime-water with the subsequent passage of a stream of carbon dioxide; the precipitate is filtered off, dried, and touched with nitric acid.

Carboluria.—In poisoning by carbolic acid, and often to a less degree after the substance has been freely used as a drug, the urine has a greenish-brown or dark brown colour, which increases on exposure to the air. This colour is due to oxidation products of some of the aromatic substances present in normal urine, which have been dealt with on p. 607 *et seq.* They are excreted in much greater quantity after the administration of phenol. Pyrocatechin and hydrochinon are especially responsible for the colour effect.

Alcaptonuria (cf. p. 607).—A phenomenon very similar to that present in carboluria is seen in certain other conditions, where an alkaline urine, as it stands in the air, takes first a brown colour at the surface, which gradually spreads through the fluid, and may finally result in the whole urine becoming nearly black. Such urine always reduces copper solutions. The phenomenon was first observed by Boedecker in 1859, and it was later ascribed by him to a substance which he called *alcapton.*

But alcapton, as already stated, is not a definite compound, and the colour phenomena are probably due to the action of oxygen upon some of the aromatic bodies present; probably, at times, upon pyrocatechin and uroleucic acid, but more often perhaps upon the homogentisic acid of Wolkow and Baumann (*vide* p. 606).

Although thought to be especially frequent in various forms of tuberculosis, alcaptonuria must not be looked upon as specifically associated with any particular diseased conditions; it indicates rather some peculiar independent changes of metabolism, and is not infrequently met with in conditions of apparent health. In one case where there was a tendency for homogentisic acid to appear in the urine, it was found that the quantity of this substance and the associated colour phenomenon might be enormously increased by administering tyrosine, and it is suggested that, when homogentisic acid or other aromatic substances appear in excess, it is due to the action of special micro-organisms on the tyrosine of the bowel.

Drug pigments.—The urine may contain purely accidental pigments due to the use of drugs, notably of rhubarb, senna, logwood, and santonin.

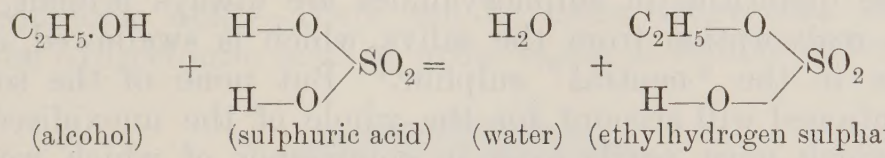
The Inorganic Constituents.

To a large extent the inorganic constituents of the urine arise directly from the food, which always contains a large excess of salts. It does not follow, however, that the bases and acids are to be found in the urine in the same combinations as when ingested, and indeed an interchange of base and acid may occur in special circumstances between the salts of the food and those of the tissue fluids. Thus, excess of potassium in the food may lead to increased elimination of sodium (Bunge). The sulphates, moreover, form an exception to the general rule of direct origin from the ingesta, very small quantities of these salts being present in a normal dietary. The urinary sulphates are derived almost entirely from proteid metabolism; a small proportion of the phosphates arising in the same way.

Sulphuric acid and other sulphur compounds.—About 80 per cent. of the total sulphur in normal human urine is present in the fully oxidised form of sulphuric acid; from 2 to 2·5 grms. of the acid,

combined as salts, being excreted per diem. Two forms of salts exist—(1) the ordinary and strictly inorganic sulphates of the form M'_2SO_4, and (2) the so-called conjugated or ethereal sulphates, which contain organic radicles; the composition of these will be clear from what follows.

If ordinary alcohol be boiled with its own bulk of strong sulphuric acid, under a vertical condenser, a crystalline product is formed which is known as ethylhydrogen sulphate, or sulphovinic acid. The composition of this is explained by the following equation:—



(alcohol) (sulphuric acid) (water) (ethylhydrogen sulphate)

By elimination of water, the ethyl radicle (C_2H_5-) becomes "conjugated" with the sulphuric acid, and a monovalent acid is formed, which yields salts of the type $M'C_2H_5 : SO_4$. On boiling such salts with hydrochloric acid, the sulphovinic acid is first liberated, and then, by absorption of water, splits up once more into alcohol and sulphuric acid.

The "conjugated sulphates" of the urine are precisely analogous salts, which undergo like decomposition when boiled with HCl. But, instead of ethyl, the radicles conjugated with the sulphuric acid are nearly always derived from aromatic precursors. In fact, as we have already seen, most of the aromatic compounds of the urine described on p. 605 *et seq.* are present as conjugated sulphates; and the proportion of the sulphuric acid present in this form depends upon the factors which increase or decrease these aromatic substances. The chief salts present, therefore, under ordinary circumstances, are those of kresyl- indoxyl- and skatoxyl-sulphuric acids.

Normally, the sulphuric acid so combined amounts to about one-tenth of the whole; nine-tenths being in the form of ordinary sulphates. An increased proportion of ethereal sulphates is found when, for any reason, there is increase of proteid putrefaction in the body, and especially in the bowel; and also when larger amounts of aromatic compounds than usual are taken by the mouth. In man they may be greatly increased during starvation, whereas, according to I. Munk, they are absent from the urine of a starving dog.

For the *detection* and *estimation* of the sulphates we rely upon the formation of the insoluble barium salt. All the sulphuric acid originally present as ordinary sulphates is precipitated as white crystalline barium sulphate, when a soluble barium salt is added to the urine, previously made acid with acetic acid. On the other hand, the barium salts of the conjugated sulphuric acids are soluble, so that when the barium precipitate, obtained as above, is filtered off, the ethereal sulphates still exist in the filtrate. But, as we have seen, they are decomposed on boiling with hydrochloric acid, splitting up into sulphuric acid and the hydrate of the conjugated radicle. It is evident, therefore, that if the above-mentioned filtrate be so boiled with hydrochloric acid, a further precipitate of barium sulphate may be obtained, the amount of which will be a measure of the proportion of ethereal sulphates present.

One-fifth of the total sulphur of the urine is present, not in any form of sulphate, but in less oxidised compounds. This fraction may be spoken of as the "*neutral sulphur*," in contradistinction to the "*acid*

sulphur" of the sulphates.[1] We have but little knowledge of the actual forms in which this neutral sulphur is excreted. As one source of the unoxidised sulphur compounds, we may look to the taurin of the bile, since it has been shown (in the dog) that when the bile is diverted from the bowel by means of a fistula, the neutral sulphur of the urine is diminished; experimental or pathological blocking of the bile duct, on the other hand, increases it.[2] A second portion is probably present in a compound or compounds analogous to cystine,[3] and in actual cystinuria the neutral sulphur is, of course, greatly increased (cf. p. 603).

Minute quantities of sulphocyanides are always present, probably owing to reabsorption from the saliva which is swallowed, and these contribute to the "neutral" sulphur.[4] But none of the sources we have mentioned will account for the whole of the unoxidised sulphur present, which must partly exist in compounds of which we have no knowledge.

To *estimate* the neutral sulphur, a small quantity of the urine is evaporated, and the residue fused with alkaline carbonates and nitrate of potassium. By this means the whole of the sulphur present is oxidised to sulphates, and these are estimated as barium sulphate. A separate estimation of the sulphuric acid originally present is made, and the amount deducted from the figure obtained, as above. The excess is a measure of the neutral sulphur, in terms of sulphuric acid.

Phosphoric acid.—The greater part of the phosphates of the urine is derived directly from those ingested with the food, but a small proportion arises from the oxidation of the nuclein, lecithin, and protagon of the tissues. The phosphates are increased by animal food, especially when this is rich in nucleo-proteids (Weintraud), and are diminished by vegetable diet. The phosphoric acid of plants is mostly present as insoluble earthy phosphates, which are not absorbed. On this account the urine of herbivora is notably poor in phosphates. In man the quantity is necessarily very variable, and ranges from 1 to 8 grms. of phosphoric acid in the urine of twenty-four hours; it commonly amounts to about 3·5 grms.

The nature of the salts present has been fully discussed in the section devoted to the chemical reaction of the urine. Part of the phosphoric acid is present in combination with lime and magnesia, but a greater part is combined with the alkalies. Some importance has been attached to a change in the relative proportion of the "earthy" and "alkaline" phosphates in diseased conditions, the estimation being made by adding ammonia to the urine and so precipitating the former. But the information so obtained may be misleading, as whatever the form of calcium or magnesium salt originally present in the urine (*e.g.* sulphates or chlorides), a precipitate produced by ammonia would contain these bases as phosphates, since an interchange of acids would take place with the alkaline phosphates. From alkaline urines magnesium ammonium phosphate (triple-phosphate) frequently separates in characteristic crystals; and in the deposit from feebly acid specimens

[1] Salkowski, *Virchow's Archiv*, 1875, Bd. lviii. S. 472.

[2] Cf. Kunkel, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 344.

[3] Goldmann and Baumann, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 254.

[4] Leared, *Proc. Roy. Soc. London*, 1870, vol. xvi. p. 18; I. Munk, *Virchow's Archiv*, 1877, Bd. lxix. S. 354.

calcium phosphate is found in star-shaped masses of fine prisms (stellar phosphate) (Fig. 58).

Pathologically, a diminution of the urinary phosphates is seen in nephritis (Purdy), and an increase is said to occur in certain nervous diseases. The phosphates may be greatly increased in diabetes insipidus.

For the *estimation* of phosphoric acid the urine is first treated with acetic acid and sodium acetate, and is then titrated with a standard solution of uranium nitrate. Ferrocyanide of potassium or cochineal tincture may be used as an indicator to mark the end point of the titration.

Hydrochloric acid.—There can be little doubt that the greater part of the hydrochloric acid of urine exists as sodium chloride, and it

FIG. 58.—*A*. Stellar phosphates; *B*. Triple phosphates.

certainly arises mainly from the common salt present in the food. The tissues and fluids of the body maintain a very constant content of sodium chloride, any excess is at once excreted, and any diminution in the supply immediately reduces the excretion. The amount in the urine depends, therefore, in normal circumstances, almost entirely upon the quantity ingested, and falls to a minimum during starvation, or when a salt-free diet is taken. Pathologically, striking alterations in the chlorides of the urine may be observed. Thus, whenever considerable exudations occur, as in pneumonic processes, or where pleuritic effusion is taking place, the consequent removal of chlorides from the blood may lead almost to a cessation of their excretion; and conversely, during the reabsorption of such exudations, the urinary chlorides may considerably increase, even when but little salt is being taken by the mouth. Apart from such exudations, fever appears to have a specific effect in pro-

moting a retention of chlorides; a fact for which we have no sufficient explanation.[1]

Upon ordinary diet, about from 6 to 10 grms. HCl is excreted per diem by a healthy adult.

To *demonstrate* the presence of chlorides, the urine is diluted, made acid with nitric acid, and mixed with nitrate of silver solution; a white curdy precipitate of silver chloride falls, which, if filtered off, is found to be soluble in ammonia.

To *estimate* the hydrochloric acid, a known quantity of silver salt is added, together with nitric acid, to a measured amount of urine, taking care that the silver is in excess. The precipitate is filtered off, and the excess of silver titrated in the filtrate with a standard solution of ammonium thiocyanate, ferrous sulphate being used as an indicator. Knowing the amount of silver added, and that left in the filtrate, the difference indicates that combined as a chloride, from which the hydrochloric acid can be calculated (Volhard's method).

Carbonic acid is found even in acid urines; some 50 c.c. being present per litre.[2] In acid urines the greater part is not in firm chemical combination, as it is driven out of solution by the passage of a stream of air. But when the urine contains abundant fixed bases, and especially when it is actually alkaline from these, considerable quantities of carbonates may be present, the urine of herbivora being exceptionally rich in these.

Nitric and nitrous acids may be present in normal urine in the form of salts, but in quite unimportant quantity.

The nitrates are derived, not from metabolism, but directly from the food; the nitrites are not present when the urine is first passed, but appear to arise always from the nitrates, as an effect of the reducing action of micro-organisms.

Silicic and hydrofluoric acids may appear in traces, simply as an effect of the presence of their salts in various foodstuffs.

Sodium and potassium.—Of sodium about 5 grms. per diem is excreted upon a mixed diet, and of potassium about half this quantity. The proportion of the latter metal is increased when the dietary is more exclusively composed of flesh, and it is raised during starvation, and in febrile conditions.[3] We have already referred to the interesting fact that the ingestion of large quantities of potassium salts may lead to increased elimination of sodium from the body, and it is this driving out of the latter essential constituent of the body-fluids which makes the consumption of common salt with the food a necessity in all cases where the diet is rich in potassium.[4]

Calcium and magnesium.—In human urine, about 0·2 to 0·4 grms. of lime (CaO) is excreted per diem.

Of the lime salts present in the food, only a small proportion is excreted by the urine. Much of the lime remains in an insoluble form, and is not absorbed at all, while of that which does enter the circulation

[1] Cf. Salkowski and Leube, "Lehre vom Harn," 1882, S. 174, 464, 465; see also Kast, *Ztschr. f. physiol. Chem.*, Strassburg, 1888, Bd. xii. S. 271.

[2] Wurster and Schmidt, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, Bd. i. S. 421.

[3] Cf. Salkowski, *Virchow's Archiv*, 1871, Bd. liii. S. 209; Munk, *Berl. klin. Wchnschr.*, 1887, S. 432.

[4] Bunge, "Lehrbuch der physiol. Chem."

a considerable fraction is re-excreted into the lower bowel.[1] The administration of dilute mineral acids, which decomposes to some extent the insoluble phosphates of the food, increases the urinary lime salts, and, conversely, when sodium phosphate is taken in large quantities, the lime may almost disappear from the urine.

Very interesting is the observation of G. Hoppe-Seyler,[2] who found that the excretion of lime salts by the kidneys is much greater during conditions of rest than during exercise, a fact which doubtless depends, in part at least, upon the effect of exercise on the excretion into the bowel.

As a general rule, the urine contains about twice as much magnesia as lime.[3] Most food-stuffs, other than milk and eggs, contain more magnesium than calcium salts. The phosphates of the former are also more soluble, and as both bases are largely present as phosphates in the food, it is to be understood that more magnesium will be absorbed and excreted. When, as during starvation, the ingestion of magnesium salts ceases, the lime is found to be in the greater proportion.

The presence of calcium in urine is easily demonstrated, and its amount determined by acidifying the fluid with acetic acid, and adding ammonium oxalate, when all the lime separates as the insoluble crystalline calcium oxalate, which may be filtered off and weighed as calcium carbonate, into which it is converted on gentle ignition. In the filtrate from this, the magnesium is precipitated as triple phosphate upon the addition of ammonium chloride, ammonia, and, if necessary, of some extraneous alkaline phosphate.

Iron.—The urine contains, as a rule, a very minute quantity of iron, and frequently no detectable trace. It has been found increased in diseases, such as pernicious anæmia, but never rises to more than a few milligrammes in the twenty-four hours. It is a remarkable fact that this metal, if present at all, is, to a large extent, precipitated in association with the pigmented crystals of uric acid, which separate when the urine is acidified with hydrochloric acid. It may be detected in the ash of large quantities of the urine, by taking a solution of this to dryness with a little nitric acid, dissolving the residue in water, and testing with potassium sulphocyanide, which gives with ferric salts a blood red coloration.

General Characteristics of the Urinary Excretives.

It might perhaps be expected that the waste products of metabolism, on leaving the body, would in general represent the simplest compounds of physiological chemistry, and would stand farthest of all removed from the complexity of the tissue proteids. That this is not entirely the case, however, will have been clear from the facts set forth in previous sections; it is, indeed, striking to observe how many of the organic excretives arise by synthetic processes from simpler precursors in the body.

There is one form of chemical change which takes an important and

[1] Voit, *Ztschr. f. Biol.*, München, 1892, Bd. xi. S. 387–397, where other references will be found.

[2] *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 161.

[3] Most analyses bear out this statement, but those of Bunge, given on p. 573, show an excess of lime.

even dominant share in the processes of constructive metabolism: that, namely, of dehydrolysis—the synthesis of larger molecules by a conjugation of smaller, associated with elimination of the elements of water. This process is known to chemists as one of "condensation." In destructive metabolism, on the other hand, the converse process of hydrolysis is an important factor.

While of most obvious importance in the physiology of plants, in which constructive metabolism starts from a lower chemical level, "condensation" is also prominent in all constructive processes of which we have any accurate knowledge in the animal organism. Being thus in general so characteristic of assimilative processes, it is remarkable how frequently dehydrolytic synthesis interrupts the course of metabolic breakdown and reappears as a final step before excretion.

We have dealt with a typical instance of this in the formation of hippuric acid from benzoic acid and glycine in the kidney, and we have seen that a like process occurs in the production of ethereal sulphates, and the conjugate compounds of glycuronic acid. We may note, too, that many substances introduced experimentally into the body undergo kindred conjugations before excretion.

The theory of the origin of uric acid and the alloxuric bases from nucleins, does not perhaps predicate any synthetic step in the production of this group of excretives; but however far-reaching may be the truth of this theory, it must be admitted that there is much reason for ascribing the origin of at least some fraction of these substances, as found in the urine, to conjugative processes in the liver and kidney. In birds there can be little doubt that uric acid arises by a synthetic change.

In the most important of the chemical changes antecedent to excretion—the formation of urea from ammonium carbonate in the mammalian liver—we have a process which I venture to think belongs essentially to the same chemical picture. Though not resulting, properly speaking, in a synthesis, the dehydrolysis which here occurs is a chemical change against the line of least resistance, and suggests an influence of the same type as that producing the synthetic results just discussed. Without misuse of the vague and somewhat discredited terms "organic" and "inorganic," we are entitled to look upon the dehydrolysis of ammonium carbonate as a return from the latter to the former category; the excretive stands physiologically on a higher level than its precursor.

To complete whatever of suggestion these considerations may contain, we may note finally the dehydrolysis which creatin suffers before excretion as creatinin. Even here we meet with a change which, for the conditions under which it occurs, is one from a more stable to a less stable substance.

It would seem that just before excretion there occurs an arrest of the normal processes of down-grade metabolism (in which hydrolysis goes hand in hand with oxidation, resulting in a series of compounds of increasing stability), and a brief return to dehydrolysis and to the type of constructive processes. It is at any rate interesting to observe that the renal excretives are as a class more complex or less stable than their immediate precursors in the body. When the urine decomposes, under the destructive influence of enzymes derived from micro-organisms, many of the precursors reappear; the urea again becomes ammonium carbonate; hippuric acid and its analogues give place to

benzoic acid, creatinin may again take up water, and uric acid is rapidly hydrolysed.

The urinary nitrogen, it will have been observed, always appears either as ammonia (NH_3), or more typically in compounds containing the derived *amido* ($-NH_2$) or *imido* ($=NH$) groups. Compounds containing the other fundamental form of organic nitrogen, the cyanogen type ($-C\equiv N$, or $-N\equiv C$), are represented only by the minute quantity of potassium sulphocyanide, which is in all probability directly derived from the saliva. Although a small proportion of the nitrogen is excreted in aromatic compounds, it is never, in human urine, present in the benzene nucleus of these, but always in side chains or accessory atomic groups within the molecule.

The carbon ring of the benzene nucleus is especially resistant to oxidation in the body, the open chain of carbon atoms, proper to substances of the fatty series, being much less so; and for the most part we find that the normal renal excretives do not reach such molecular size as to contain as many as six carbon atoms, unless they contain the aromatic nucleus. As illustrating the degree of molecular complexity found in the organic urinary compounds, we may remember that the molecular weight of urea is 60, that of creatinin is 113, of uric acid 168, and of hippuric acid 181. The intact renal epithelium, it is true, passes substances, such as the pigments, the molecular weight of which is much greater than the above, but only in small quantities.

Comparative Chemistry of the Urine.

In mammals, amphibia, fishes, and in certain molluscs, urea is the chief end-product of nitrogenous metabolism. In birds, reptiles, and arthropods, on the other hand, the nitrogen is excreted mainly in the form of uric acid. In spiders and in some few other groups of invertebrates, the chief excretive has been shown to be guanin.

A study of the renal function, from a comparative point of view, offers one aspect of great interest and some difficulty, to which Sir William Roberts has called attention. It is remarkable that the wide differences in the nature of the renal excretion in mammals and the *Sauropsida* respectively, should yet be associated with almost complete identity in the anatomical structure of the kidney. The kidney of the bird has a glomerular mechanism identical with that of the mammalian organ, and the same tubular arrangement of a secretory epithelium; and yet practically the sole function of the organ of the bird, in contrast to the remarkably complex duties performed by that of the mammal, is to secrete quadriurates. "The chlorides, phosphates, and sulphates, the lime and magnesia salts, the pigments and the large volume of water—all of which figure as prominent and even essential components of mammalian urine—are either wholly absent from the urine of birds and serpents, or are only present in such minute traces as might be derived from the lubricating mucus and epithelial débris with which the secretion is incidentally admixed" (Roberts).

The physiological differentiation, whereby soluble urea takes the place of insoluble uric acid, in accordance with the needs of an organism excreting a liquid urine, is now known to occur quite at the final stages of metabolism. It is almost certain that, in the main, the

waste products which leave the tissues are the same in birds and mammals. In the liver of the former these products are prepared for excretion by a change into the form of uric acid, while in the latter the hepatic influence produces urea. There is a great preponderance of experimental evidence to show that when uric acid is administered to mammals it is converted into urea before excretion, and that when urea is given to birds the converse change occurs. The contention of Haig, that when uric acid is taken by the mouth (in man) it is excreted unchanged, is not supported by other observers.

As to the small quantity of uric acid found, nevertheless, in the urine of mammals, if we accept the theory of its exclusive origin from nucleins, it is clear that we cannot look upon it as in any sense physiologically akin to the main part of the normal excretion of birds, for this must represent the waste nitrogen of the tissues as a whole. But this theory apart, the view is plausible, and indeed it cannot be said to be yet disproved, that we have in the mammalian uric acid a vestigial relic of the earlier type of excretion—"something analogous with the vermiform appendix, the ductus arteriosus, or the ear-point." The actual proportion present in the urine of different mammals is very variable. In most animals the relative amount is less than in man, but, except occasionally in the cases of the cat and dog, it has never been found to be absent. The presence of the small amount of uric acid in the urine of mammals is paralleled by the existence of minute quantities of urea in that of birds and reptiles.

Creatinin has been found wherever looked for in the urine of various species of mammals, but is said to be absent from the excretion of birds.

Hippuric acid is represented in birds by the analogous compound, ornithuric acid, which is a condensation product of benzoic acid with diamidovalerianic acid. An aromatic acid, apparently peculiar to the urine of dogs, is known as kynurenic acid, and has the composition of an oxychinolin-carboxylic acid ($OH.C_9H_5N.COOH$).

The large proportion of hippuric acid in, and the absence of ammonium salts from, herbivorous urine, have been shown in previous sections to be, in common with the alkaline reaction of the fluid and its richness in salts, a direct effect of diet.

Of the urinary pigments in the lower animals we have no accurate knowledge.

It is impossible at present, owing to the wide gaps in our knowledge, to take any broad view of the comparative chemistry of the urine. A series of analyses are much needed, from the results of which we could form some judgment as to the line of evolution which has led from the simple renal excretions of the invertebrates to that most complex of physiological fluids—mammalian urine.[1]

[1] See on the subject of the comparative chemistry of the urine, Rywosch, *Wien. med. Wchnschr.*, 1893, Nos. 47 and 48.

THE MECHANISM OF THE SECRETION OF URINE.

By Ernest H. Starling.

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Theories of Urinary Secretion.

In all the organs of the body whose functions have been investigated by physiologists, it has been found that a difference of function is invariably associated with a difference in structure, so that the interdependence of function and structure has become an axiom. We are therefore justified in founding theories concerning the physiological function of an organ on a purely anatomical study of its structure, although the complete establishment of such theories must ultimately be afforded by physiological investigations.

The kidney differs from all other secretory glands, in the fact that at the blind end of its tubulus we find a structure—the glomerulus—where the vascular capillaries abut directly on the lumen of the tubulus, without the interposition of any lymph space. Ever since the publication of Bowman's paper on the Malpighian bodies of the kidney, these have been looked upon as the essential source of the watery constituents of the urine.

Theory of Bowman.—Bowman,[1] who founded his theory of urinary formation exclusively on the anatomical structure of the kidney in various animals, concluded that "as the tubes and their plexus of capillaries were probably the parts concerned in the secretion of that portion of the urine to which its characteristic properties are due (the urea, lithic acid, etc.), the Malpighian bodies might be an apparatus destined to separate from the blood the watery portion."

The following are the grounds on which Bowman based this hypothesis:—

(*a*) *That the tubes are secretory.*

(1) The extent of surface obtained by the involutions of the tubules.

(2) The fact that the lining membrane of the tubules is formed by thick epithelial cells, similar to those found on the secreting surface of all true glands.

[1] *Phil. Trans.*, London, 1842, p. 57.

(3) The capillary network surrounding the uriniferous tubes is the counterpart of that investing the tubes of the testis, allowance being made for the difference in the capacity of these canals in the two glands.

(*b*) *That the Malpighian bodies differ from the secreting parts of true glands.*

(1) The Malpighian bodies comprise but a small part of the inner surface of each kidney, there being but one to each tortuous tube.

(2) The epithelium immediately changes its characters as the tube expands to embrace the tuft of vessels.

(3) The blood vessels, instead of being on the deep surface of the membrane, "pass through it and form a tuft on its free surface."

(4) The peculiar arrangement of the vessels in the Malpighian tufts is clearly designed to produce a retardation of the blood through them, while the orifice of the tubule is encircled by cilia in active motion directing a current towards the tubule, so tending to remove pressure from the free surface of the vessels and to encourage the escape of their more fluid contents. "Why is so wonderful an apparatus placed at the extremity of each uriniferous tube, if not to furnish water to aid in the separation and solution of the urinous products from the epithelium of the tube?"

The appearance of this paper fell at a time when, led by Ludwig, Helmholtz, and du Bois Reymond, physiologists were endeavouring to replace the misty "vitalistic" conceptions which had until then prevailed, by an accurate comparison of vital phenomena with their physical or chemical counterparts, and seeking to establish physiology as an exact experimental science on a par with physics. It was impossible, therefore, that the views of Bowman, devoid as they were of experimental foundation, should remain unchallenged.

Theory of Ludwig.—In 1844, Ludwig[1] put forward his well-known mechanical theory, for the establishment and testing of which a large volume of work has been done, the greater part under the direction of Ludwig himself. According to this theory, all the energy for the secretion of urine is ultimately derived from the heart-beat. In consequence of the high pressure obtaining in the capillaries of the glomeruli, a fluid is filtered through, containing all the constituents of the urine in very dilute solution. This dilute solution passes down the tubules, and in its passage undergoes changes, in consequence of diffusion between it and the fluid (lymph) surrounding the tubules. Since water will always pass from a dilute to a more concentrated fluid, and since the glomerular filtrate is, according to the theory, poorer in solid constituents than the serum, water will pass from urine to lymph, and the urine will become more concentrated until it acquires the normal characters of urine.

In this theory of Ludwig there are three distinct propositions to be investigated. These are—

1. That the secretion of water is a purely mechanical process,

[1] Wagner's "Handwörterbuch," 1844, Bd. ii. S. 637; "Lehrbuch der Physiologie," Aufl. 2, 1858, Bd. ii. S. 373.

depending only on the blood pressure in the glomerular capillaries and the permeability of the filtering membrane.

2. That this dilute urine is concentrated in the tubules by giving up its water to the surrounding lymph, in consequence of differences of concentration between the glomerular filtrate and the lymph.

3. That all the urinary constituents are turned out of the blood through the glomeruli (*i.e.* with the water) in dilute solution.

In discussing the experimental data bearing on these propositions, we shall find that only in the first part of the theory are the experimental facts consonant with Ludwig's hypothesis, and that it is impossible to explain the formation of normal urine without assuming the active intervention (*i.e.* the performance of work) by certain of the living elements of the kidney in the process. In this case, as in so many others in physiology, the "how" of the cellular activity is at present absolutely unknown to us, although we may confidently expect, with the advance of the science, to be able to trace the manner in which the cell utilises the energy of its food for this special purpose.

Secretion of water.—One of the strongest facts in favour of Ludwig's hypothesis is the indubitable connection which exists between the circulation through the kidney and the amount of urine, *i.e.* of water, secreted. It is evident that a mechanical filtration or separation of the watery and crystalloid constituents of the blood in the glomeruli must depend on two factors—

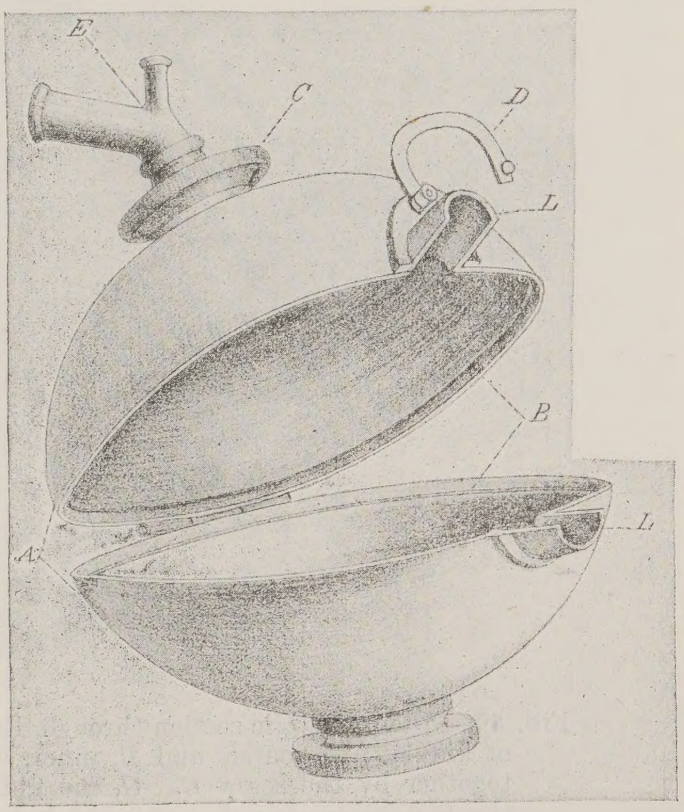

FIG. 59.—Roy's oncometer. (For explanation of lettering, see next figure.)

1. The difference of pressure between the blood in the glomerular capillaries and the urine in Bowman's capsule. Since under normal circumstances there is a free outflow of urine from the capsule by means of the tubules, we may regard its pressure as practically nil, so that the only changeable factor in the process will be the blood pressure in the capillaries.

2. The rapidity of the blood flow through the glomeruli must also have some influence on the filtration, as this process will go on the more readily the more often the fluid presented to the filter is renewed. As a rule, the changes that raise the pressure in the capillaries also increase the velocity of the blood through them, so that it becomes difficult to dissociate the part played by each factor in influencing the urinary secretion.

If we consider the manner in which changes in the glomerular circulation are brought about, we see that it may be affected by changes either in the general blood pressure or in the calibre of the smaller

arteries. The pressure in the glomerular capillaries will be raised and the velocity of the blood increased—

1. By a rise of general blood pressure. This may be due to—

(*a*) Increased force or frequency of the heart beat;

(*b*) Constriction of vascular areas in other parts of the body.

2. By dilatation of the renal arterioles, the general blood pressure remaining constant.

3. By obstruction of the renal vein. In this case the velocity will be diminished.

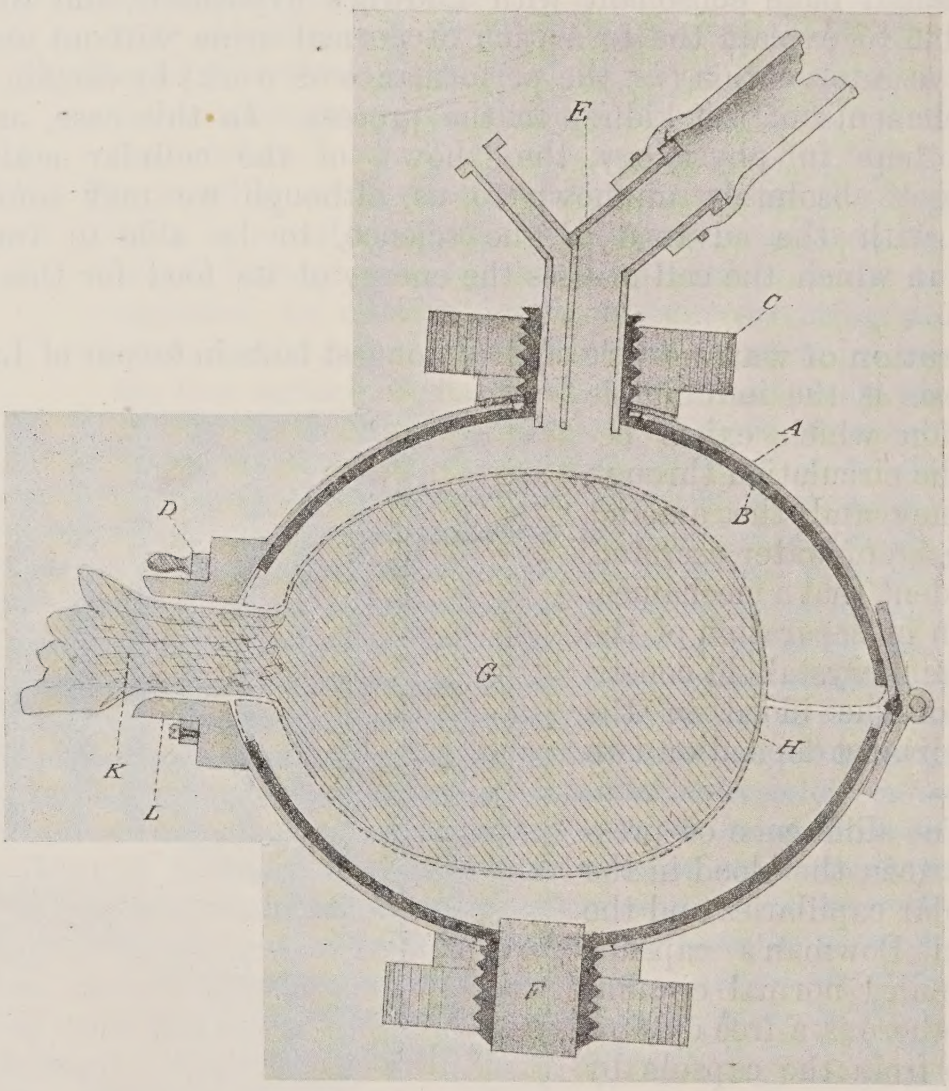

FIG. 60.—Diagrammatic section through Roy's oncometer, to show position of kidney. *A*, outer, and *B*, inner, brass capsules. These are fixed together by the screw *C*. *G*, the kidney. *D*, a clamp for fastening the two halves together after the kidney has been inserted. *K*, renal vessels and ureter.

The pressure and velocity in the glomerular capillaries will be diminished—

1. By diminished general blood pressure, which may arise from a weakening or slowing of the heart-beat, or from dilatation of vascular areas in other parts of the body.

2. By constriction of the vessels in the kidney itself.

Methods.—In the earlier researches[1] on the connection between the renal circulation and the flow of the urine, the observers had to content them-

[1] Max Hermann, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1859, Bd. xxxvi. S. 349; 1861, Bd. xlv. S. 317; Ustimowitsch, *Arb. a. d. physiol. Anst. zu Leipzig*, 1870.

selves with a measurement of the general blood pressure, and could only obtain direct evidence as to the local changes in the renal circulation by inspection of the kidney. It was not until the ingenious application of plethysmographic methods to the kidney *in situ*, by Roy,[1] that we could obtain a precise and quantitative estimate of the changes produced on the circulation through this organ by the measures employed by the older observers.

Roy's instrument for registering changes in the volume of the kidney consists of two parts, in one of which, the oncometer, the kidney is placed, while another, called the oncograph, serves as the recording part of the apparatus. The oncometer consists of two halves hinged together, each of which is formed of two metal capsules screwed together by the screw *C*, and holding between them the membrane *H*. The two halves thus form a box. When the two halves are approximated, the box is closed except at one point *K*, opposite the hinge, where there is an opening to allow the passage of the renal vessels and nerves, and the ureter to the kidney, which is placed within the box. During use, the space between the membrane and the metal box is filled with warm oil through the opening in the screw. The opening in one-half is then closed with a plug, while the other communicates by a tube *E*, with the oncograph. It is evident that any change in the volume of the kidney will be communicated to the oil between the membrane and the capsule, oil being driven out into the tube *t*, when the kidney swells, and being sucked in directly any shrinking of the kidney occurs. The oncograph, which is practically a piston-recorder, in which the piston is made oil-tight by resting on a loose peritoneal membrane tied round the tube, serves to register the amount of oil driven out or sucked into the oncometer, and therefore at the same time the changes in the volume of the kidney.

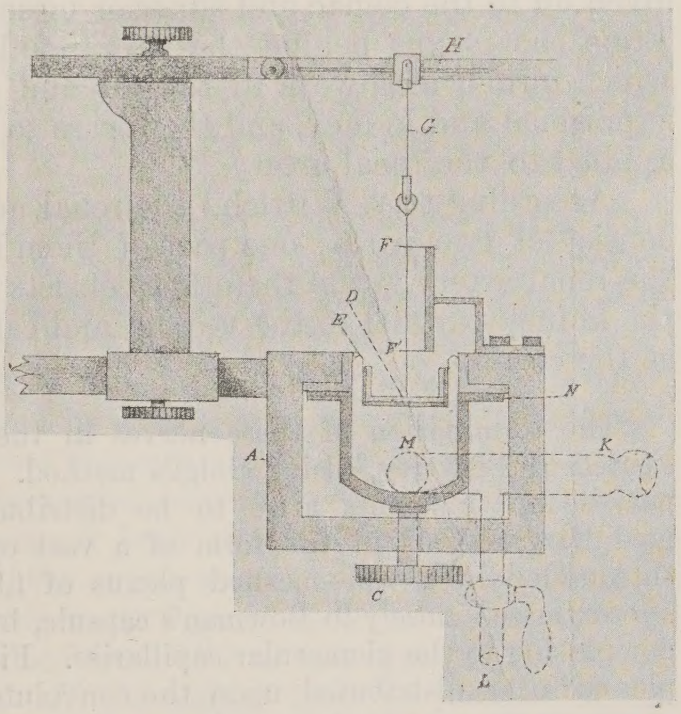

FIG. 61.—Roy's oncograph. Diagrammatic section. The cylinder *M* is filled with oil, and communicates by the tube *K* with the oncometer. Changes in the height of the oil are communicated by the piston *D* to the lever *H*, the excursions of which serve therefore as an index of the changes in volume of the kidney.

A simpler and more efficacious form of oncometer, in which air instead of oil is used, has been devised by Schäfer[2] for the spleen, but is equally applicable to the kidney. A description of it will be found in the section dealing with the physiology of the circulation.

Nerve supply.—Before discussing the effects of various operative procedures on the circulation of the kidney, it will be necessary to say a few words concerning the nerve supply to this organ, since its vessels, like those of all other parts of the body, are under the direct control of the central nervous system.

The gross distribution of nerves to the dog's kidney has been the subject of a careful investigation by Nöllner[3] in Eckhard's laboratory.

[1] *Journ. Physiol.*, Cambridge and London, vol. iii. p. 205. [2] *Ibid.*, 1896, vol. xx.
[3] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1869, Bd. iv. S. 139.

The course taken by the nerves is very variable. The nerves are derived from the sympathetic chain. From the ganglia lying on the head of the thirteenth rib, or from the cord immediately below this, is given off a large nerve (larger than the continuation of the sympathetic chain), which perforates the crus of the diaphragm, and is called the large splanchnic nerve. Between this ganglion and the next two or three ganglia below, are given off three or four smaller filaments, known as the small splanchnic nerves. (It must be noted that this terminology is not comparable with that employed in human anatomy, where the term splanchnics is confined to the nerves given off by the sympathetic chain while in the thoracic cavity.) These large and small splanchnics form a plexus situated behind the suprarenals, and from which filaments are given off to the cœliac and superior mesenteric ganglia and solar plexus. From the plexus behind the suprarenals arise a number of filaments, which form a meshwork in the fat and connective tissues between the suprarenal and kidney, and then pass to the kidney around, and closely applied to, the renal artery.

According to v. Wittich,[1] the renal nerves in the rabbit, dog, and man consist of two parts; one part of them forms a plexus closely investing the renal artery, while the other consists of several filaments which enter the kidney parallel to the vessels, and can be traced along these as far as the cortex.

The termination of these nerves in the kidney has been recently investigated by Berkeley,[2] using Golgi's method. He finds that from the vascular nerves fine filaments arise to be distributed throughout the cortical and medullary regions in the form of a vast open network. The glomeruli are surrounded by a wide-meshed plexus of fibres, having terminal end knobs, approximated closely to Bowman's capsule, but not penetrating that membrane, nor passing to the glomerular capillaries. Fibres also pass off from the vascular plexus to be distributed upon the convoluted tubes, with terminations which penetrate the membrana propria of the tube, presumably to enter the cement substance between the epithelial cells. Berkeley looks upon these latter nerves as probably secretory in function.

With regard to the connection of the renal nerves with the central nervous system, Bradford[3] has shown that, so far as the efferent nerves to the renal vessels are concerned, these leave the spinal cord through the anterior roots. Most of the fibres are contained in the eleventh, twelfth, and thirteenth dorsal nerve roots.

Influence of blood flow on secretion of urine.—We are now in a position to consider the influence exerted by changes in the circulation through the kidney on the secretion of urine. It must be remembered that a rise of general blood pressure does not necessarily carry with it an increased pressure in the glomerular capillaries or an increased blood flow through the kidney. Thus, under many conditions, a rise of general blood pressure is brought about by a constriction of all the visceral arteries, including those of the kidney, and such constriction is more than sufficient to counteract the effects of the increased blood pressure. If we take a tracing of the kidney volume, for instance, in asphyxia, we

[1] Königsberger, *Med. Jahrb.*, Wien, 1860, Bd. iii. S. 52 (quoted from Heidenhain in Hermann's "Handbuch").

[2] *Journ. Path. and Bacteriol.*, Edin. and London, 1893, vol. i. p. 406.

[3] *Journ. Physiol.*, Cambridge and London, 1889, vol. x. p. 358.

find, coincident with the rise of general blood pressure, a marked shrinking of the kidney. On the other hand, a dilatation of the renal vessels may be ineffectual to produce an increased flow through this organ, if at the same time there is a large fall of general blood pressure due to dilatation in other parts of the body. We may consider, in the first place, experiments in which the chief change has been in general blood pressure. It is found that, if the aortic pressure sinks below 40 mm. Hg, the urinary secretion stops absolutely. So long as the aortic pressure is above this height, the secretion is more or less proportional to the pressure, and changes with the changes in this pressure. Thus, if we stimulate the vagus in the neck, using currents sufficiently strong to produce a slowing of the heart-beat and a fall of blood pressure, there is a shrinking of the kidney and a diminution in the urinary flow (Goll). That this diminution in the flow is directly conditioned by the change in blood pressure due to the cardiac inhibition, is shown by the fact that stimulation of the vagi below the diaphragm is without effect on the urine (Eckhard).

We may also alter the aortic pressure by bleeding the animal to a considerable amount, and later on reinjecting the blood so withdrawn. It is found that after the bleeding, while the blood pressure is diminished, the flow of urine is also lessened, but the flow increases when the blood pressure is raised by reinjecting the blood which had been withdrawn.

If the aortic pressure be raised by ligaturing a number of the larger arteries, the increased flow of blood through and the increased pressure in the kidneys are attended with increased secretion of urine. Thus in one experiment in which Goll[1] ligatured both carotids, both femorals, and both ascending cervical arteries, the urine was increased from 8·7 grms in 30 minutes before the ligature, to 21·2 grms. after the ligature, while the pressure in the aorta was raised from 127 to 142 mm. Hg.

Division of the spinal cord.—If the spinal cord be divided in the upper cervical region, the result is a great fall in general blood pressure, which may be as low as 30 to 40 mm. Hg. In all cases where the blood pressure falls below 40 mm. Hg, the flow of urine is absolutely abolished. Since the renal vessels, like those of all other parts of the body, are kept in a condition of tone by impulses descending from the vasomotor centre in the medulla, division of the path of these impulses must cause a relaxation of the renal vessels, which by itself would tend to occasion increased blood pressure in the glomeruli. As a result of the section, however, the vessels all over the body are relaxed, so that the capacity of the vascular system is increased and the peripheral resistance diminished, both factors concurring to produce the large fall of pressure observed. This fall of pressure is more than sufficient to counterbalance the local renal dilatation, so that there is diminished blood flow through the kidney, as is shown by the marked shrinking of the oncometric curve of the kidney on section of the cord.

Stimulation of the cord.[1]—If the peripheral end of the divided cord be stimulated with an induction current, universal constriction of the blood vessels and a large rise of blood pressure are produced. This, however, is incompetent to bring back the urinary flow which has been abolished by the previous section, since the renal vessels concur in the general constriction, and the kidney shrinks still further in spite of the raised blood pressure. If, however, this local constriction be prevented by previous

[1] *Ztschr. f. rat. Med.*, 1854, N. F., Bd. iv. S. 86 (quoted by Heidenhain).

division of all the renal nerves, stimulation of the cord causes a large expansion of the kidney and brings back the urinary flow.

Influence of the splanchnics.[1]—The effects of stimulating the splanchnic nerves are very similar to those obtained from the stimulation of the cord. As in the latter case, a large rise of general blood pressure is produced, but the constriction of the renal vessels more than counteracts the effects of this rise, so that the kidney shrinks and the flow of urine is diminished or abolished. The effects of dividing the splanchnics vary in different animals. In the rabbit, where, in consequence of the extent of the vascular area supplied by this nerve, a considerable fall of general blood pressure is produced, no increase in the urinary secretion is observed. In the dog, on the other hand, the lasting effect on the aortic pressure is insignificant, so that the relaxation of the kidney vessels caused by the section induces a largely increased flow through this organ, and a marked increase in the flow of urine.

Influence of renal nerves.—Division of the renal nerves on one side causes vasomotor paralysis in the organ of that side. The kidney therefore swells, and the flow of urine is increased. The swelling and secretion is still further increased if the general blood pressure be raised by stimulation of the splanchnics or spinal cord. Stimulation of the renal nerves causes constriction of the vessels and diminished flow of urine.

Bradford[2] has brought forward evidence to show that vaso-dilator fibres run to the kidney with the constrictors, in the eleventh, twelfth, and thirteenth dorsal nerve roots. If the anterior roots of these nerves be stimulated with induction shocks, repeated at the rate of one per second, the effect is often a marked swelling of the kidney without any rise of blood pressure sufficient to account for the enlargement. A similar active dilatation of the vessels may be brought about reflexly by stimulating the posterior roots of these nerves. We have no direct experimental evidence as to the influence of this active vascular dilatation on the renal secretion, although it is extremely probable that a similar condition is the chief factor in the production of the extreme hydruria met with in hysteria and other nervous affections.

Constriction of renal artery.—In some of the earliest researches on the connection between the blood flow through the kidney and the urinary secretion, it was sought to affect the circulation by direct mechanical constriction of the renal artery. Hermann,[3] who carried out experiments of this nature under Ludwig's guidance, showed that when the artery was constricted to a considerable extent, the result was a diminished flow of urine. If the constriction were carried so far that the circulation of the kidney was entirely stopped, the flow of urine instantly ceased. So far these results are those one would expect on the filtration hypothesis. It is found, however, that the flow of urine is not restored at once on relieving the constriction, and that after a few minutes' total cessation of the renal circulation, more than an hour may elapse between the restoration of the circulation and the recommencement of the secretion. We have seen that in the case of lymph formation, where a process of filtration almost certainly comes into play, a temporary ischæmia increases the permeability of the vessel wall, so that, on the

[1] See especially Eckhard, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1869, Bd. iv. S. 132, and 153–193.

[2] *Loc. cit.*

[3] *Loc. cit.*

subsequent restoration of the blood flow, the amount of lymph transuded is greater than before the ligature. In the kidney the reverse is the case. A temporary ischæmia abolishes the flow for a considerable period after the obstruction has been relieved. This fact shows that, for the normal production of urine, the integrity of the living cells between the blood and Bowman's capsule is necessary; but I do not think that it can be looked upon as definitely proving the active co-operation of these cells in the process. In the kidney we have two layers of cells, the vascular endothelium and the glomerular epithelium, intervening between the blood and urinary tubule, and we have no evidence to guide us as to the effects of temporary ischæmia on the glomerular epithelium. We know that in a certain sense it becomes more permeable, inasmuch as the urine which is first secreted after the restoration of the circulation contains albumin, which may be traced on its way through the glomerular epithelium into the capsule. But this fact in itself might tend to impede the flow of water through the glomerular membranes.

Ligature of renal vein.—In the case of lymph formation, a rise of venous pressure tends to increase the amount of lymph produced. In the kidney, ligature of the renal vein stops the flow of urine at once, although it must send up the pressure in the glomerular capillaries to a height approaching that of the renal artery. Now, in this case there are three factors which might be concerned in causing the cessation of secretion: the blood flow through the kidney is checked; the cells of the glomerular epithelium are asphyxiated; and the engorgement of the renal veins causes the interlobular veins to swell up and press on the adjoining collecting tubules. Heidenhain lays most stress on the first factor, and, relying mainly on this experimental result, concludes that the chief agent in exciting glomerular activity is not the blood pressure in the glomerular capillaries, but the rapidity of the flow through the capillaries. On the other hand, Ludwig has shown that the effect of the swelling of the interlobular veins is to obstruct the urinary tubules; and he looks upon the cessation of flow as entirely due to this mechanical obstruction. It is impossible at present to decide which of these explanations is correct, or indeed whether all of them may not be involved.

Action of diuretics.—Since the main office of the kidney is to assist in maintaining the normal constitution of the blood by freeing it from the waste products of tissue metabolism, we should expect it to react and to be sensitive to slight changes in the composition of the blood. As a matter of fact, we find that such is the case, and that the easiest way to excite the urinary flow is by altering the composition of the blood, through the administration of large quantities of water, or of certain drugs which are known as diuretics. Of these bodies the only ones we need discuss are the large class known as saline diuretics and the drugs caffein and digitalis.

Saline diuretics include practically all crystalloid substances, which can be injected into the blood in considerable quantities. As examples, we may cite urea, dextrose, sodium chloride, potassium nitrate, sodium acetate, etc. If these bodies be injected into the blood, a very copious secretion of urine is soon evoked, even if, previously to the injection, the secretion had been at a standstill. In experiments on the excised kidney, it has in most cases been found necessary to add urea or some other substance of this group to the defibrinated blood used for the

artificial circulation, before any secretion could be obtained.[1] On inquiring into the mode of action of these bodies, we find that their injection is followed by a slight rise of blood pressure accompanied with a marked expansion of the kidney, and this expansion lasts throughout the period of increased urinary flow. These effects are observed even after all the renal nerves have been severed, so far as is practically possible, and it has therefore been concluded that the changes in volume of the kidney must be due to the substances acting either upon some peripheral vasomotor mechanism, or even more directly upon the blood vessels themselves. Since the increased secretion of urine is coterminous with the increased blood flow through the kidney, it is natural to place these two events in the relation of effect and cause. To this conclusion it has been objected that one may frequently observe an absolute standstill of secretion, with a high aortic blood pressure changed into a copious secretion by the injection of one of these bodies, although the blood pressure has been practically unaltered. Heidenhain and others with him, therefore, look upon the action of these bodies as secretomotor. Against the specific secretomotor action, either of urea or of the salines, the following arguments may be brought forward. V. Limbeck[2] has shown that the power of these bodies to induce urinary secretion on injection into the blood stream is proportional to their power of attracting water (*Wasseranziehungsvermögen*), and is thus a function of their molecular weights. Now it has been proved[3] that the result of injecting these bodies into the blood is to cause an active flow of water from the tissues into the blood, which therefore becomes diluted to an extent varying with the osmotic pressure of the substances injected. The final effect, therefore, is the same as if a solution of the substance isotonic with or normal to the blood had been injected into the circulation, and a condition of hydræmic plethora thus induced. We know that a condition of hydræmic plethora is associated with dilatation (especially of the visceral vessels), general rise of capillary and venous pressures, and increased rapidity of blood flow. The fact that the diuretic action of these bodies is proportional to their osmotic pressures, implies that it is also proportional to the hydræmic plethora produced by the injection; and it seems probable, therefore, that the plethora is the chief agent in causing, first, the vascular changes in the kidney, and secondly, the diuresis. If these bodies acted as specific stimulants of the kidney, we should expect the increased flow of urine to continue until all the substance injected had been excreted. Such, however, is not the case. The diuresis comes to an end when only a small amount of the injected substance has been excreted, and lasts little or no longer than the hydræmic plethora which accompanies it.

Of the other diuretics, the action of two, caffein and digitalis, has been very fully investigated. If half a grain of caffein be injected into a vein, the kidney after a few seconds diminishes in volume, and the flow of urine is lessened or entirely arrested. This contraction soon passes off, and is followed by a rapid expansion, which is more considerable and lasts much longer than the preceding contraction. Simultaneously with the beginning

[1] M. Abeles, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1883, Bd. lxxxvii.; I. Munk, *Virchow's Archiv*, 1886, Bd. cvii. S. 291; *ibid.*, S. 187; and I. Munk and Senator, *ibid.*, 1888, Bd. cxiv. S. 1.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxv. S. 69.

[3] V. Brasol, *Arch. f. Physiol.*, Leipzig, 1884, S. 211; Starling, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvii. p. 30; Leathes, *ibid.*, 1895, vol. xix. p. 1.

of the expansion, the urinary flow recommences and becomes much more rapid than it was previously to the injection of the drug. On the general blood pressure the injection of caffein causes an initial slight fall, followed by a return to normal or a little above normal. In this case we seem to be dealing with a drug, the most important action of which is on the renal vessels, and it is probable that the increased pressure in and flow through the glomerular capillaries induced by the drug is largely responsible for the augmented flow of urine. According to von Schröder,[1] it is possible, by the administration of chloral, to abolish the vaso-dilator effect of caffein in rabbits without destroying the diuretic action of the drug; but too much reliance cannot be placed on this statement, since the volume of the kidney was not measured in this observer's experiments.

The effect of digitalis is rather more complex. It slows and strengthens the cardiac beat, and at the same time constricts the smaller arteries of the body, so that the arterial pressure is raised. In heart disease the result of the improved working of the cardiac pump is to relieve the venous pressure, increase the arterial pressure, and so bring about an improved blood flow through the kidney. In such cases, therefore, digitalis acts as a powerful diuretic. In the healthy animal the effect of this drug is more doubtful. It causes a constriction of the renal vessels and therefore a shrinking of the kidney. Under certain circumstances, however, it does exert an appreciable influence in causing diuresis, which we may either explain, with Bradford and Phillips,[2] as due to a direct action of the drug on the renal epithelium, or to the fact that the rise of blood pressure more than counteracts the renal constriction, so that there is an increased blood flow and pressure in the glomerular capillaries.

Effects of ligature of the ureter.—If we are to look upon urine as a filtrate, the amount of it must vary as P—p, where P represents the pressure in the glomerular capillaries, while p represents the pressure at the beginning of the urinary tubule. So far, we have only considered the effects of altering P, and have seen that, in the majority of cases at any rate, the secretion of urine rises and falls with this pressure. Under normal circumstances p is so small that it may be neglected, but we ought to be able to diminish the flow of urine by increasing p. If the ureter be obstructed by connecting it with a mercurial manometer, it will be found that the mercury in the manometer rises quickly to 10 or 20 mm. Hg, and then more slowly until, in the dog, it may attain the height of 50 or 60 mm. Hg, at which pressure the mercury column remains stationary. The pelvis of the kidney and the ureter above the ligature are now strongly distended; the kidney is swollen, and a marked œdema is soon observed extending to the perinephritic tissues, while the lymphatics of the hilus are distended with clear fluid. Some hours later, hæmorrhages are found in the fatty capsule and in the pelvis and ureter. Ludwig interpreted these results as determining the conclusions he had already drawn from the effects of section of the spinal cord, *i.e.*, that, for the production of urine, a certain minimum difference of pressure P—p is necessary, and that the difference might be reduced below this limit either by diminution of P or by augmentation of p.

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 39; 1888, Bd. xxiv. S. 85.

[2] *Journ. Physiol.*, Cambridge and London, 1887, vol. viii. p. 117.

Heidenhain, however, points out that we have no right to conclude that the secretion of urine has ceased when the mercury column no longer rises. This stage in fact corresponds merely to the point at which the continued secretion of urine is balanced by the reabsorption of the urine from the tubules, in consequence of the abnormal pressure within them.

It must be confessed that we have no very definite evidence that such a reabsorption takes place. It is true that the kidney becomes œdematous in consequence of the ligature, but the œdema fluid was stated by Ludwig to consist of lymph and not of urine; and it has been shown that increased pressure in the urinary tubules causes them to press on the adjoining veins, so that the escape of blood from the kidney is hindered, and ordinary œdema results. Fresh investigations on this matter are much to be desired, since the only analyses we have of the œdema fluid and retained urine are those of Hermann, one of the earliest observers on the subject. The urine, which is secreted under pressure and which distends the pelvis and ureter, is light in colour, of low specific gravity, and contains very little urea. If, after some time, the ligature round the ureter be relaxed, the result is at once a copious secretion of watery urine. In man a similar fluid is well known to be excreted in cases where there is a chronic obstruction of the ureter.

The concentration of the urine.—We have now to consider the second part of Ludwig's theory, according to which the dilute urine transuded through the glomeruli is concentrated on its passage down the tubules, by the absorption of its water. This absorption takes place in consequence of the fact that the lymph surrounding the tubules is more concentrated than the urine. A cogent objection to this hypothesis was raised in 1859 by Hoppe (Hoppe-Seyler), who showed that, if urine were separated by an animal membrane from blood serum of the same animal, there was a flow of water from serum to urine.[1] The tendency of this urine, therefore, in passing down the urinary tubules, would have been to become more dilute, in consequence of osmotic interchanges between it and the serum. At this time our knowledge of the factors and forces involved in the interchange of water and substances in solution across animal membranes was meagre and inexact; and it is only quite recently that we have acquired the necessary data for testing the truth of Ludwig's hypothesis and the fitness of Hoppe-Seyler's objections.

Pfeffer[2] showed that the osmotic attraction of any solution for water might be determined by measuring its osmotic pressure, and first pointed out how enormous these pressures were in the case of even relatively dilute salt solutions. Van t' Hoff later on pointed out that the osmotic pressure of a solution was proportional to the number of molecules this contained, and was therefore a colligative property (Ostwald), like certain other properties of solutions—such as the diminution of the freezing point and of the vapour tension and the elevation of the boiling point.

Since these properties of a solution are proportional to one another, we need only know one to determine any of the others. This fact is of importance when we wish to determine the osmotic pressure of animal fluids, since we can substitute for the difficult and inexact determination

[1] *Virchow's Archiv*, 1859, Bd. xvi. S. 412 (quoted by Heidenhain).
[2] "Osmotische Untersuchungen," Leipzig, 1877.

of osmotic pressures by Pfeffer's method a determination of the freezing-point of the solution. As van t' Hoff has shown, if Δ is the depression of freezing-point and T the absolute freezing-point of the solvent (*i.e.*, for water, 273°, and w the latent heat of fusion of ice = 79 cal.), then the work A can be reckoned from the following formula:—

$$dA = \frac{\Delta w}{T} \times dv.$$

Thus for 1 per cent. solution of cane-sugar ($\Delta = \cdot 055$)

$$dA = \frac{\cdot 055 \cdot 79}{273} \times dv.$$

To reduce this result to gravitation units we must multiply by 424, and we thus find that to separate the volume dv of pure water as ice from 1 per cent. cane-sugar solution, a force is necessary equal to the pressure of a column of water of $\frac{\cdot 055 \times 79 \times 424}{273}$ metres in height.

A depression of $\Delta = -1°$ corresponds therefore to an osmotic pressure of $\frac{79 \times 424}{273}$; that is to say, to 122·7 metres of water. We have therefore to multiply Δ by 122·7, in order to obtain the osmotic pressure in metres of water of any solution.

Now it is evident that, according to Ludwig's hypothesis, the osmotic pressure of the urine might attain to but could never exceed that of the blood plasma. On estimating the osmotic pressures of these two fluids, we find that, under normal circumstances, the osmotic pressure of the urine is considerably greater than that of the blood, so that work must have been done in the separation of this concentrated fluid from the more dilute blood plasma. Dreser[1] has estimated this work in a case in which, during one night, 200 c.c. of urine were secreted with $\Delta = 2 \cdot 3$. This was separated by the kidneys from the blood with $\Delta = \cdot 56$. In the production of this fluid Dreser finds that the work done by the kidney amounts to 37·037 kilogramme metres. This figure by no means represents the maximum force which can be exerted by the kidney. From a cat which had been deprived of water for three days, Dreser drew off some urine with $\Delta = 4 \cdot 72$ C. The blood at the same time had an osmotic pressure corresponding to $\Delta = 0 \cdot 66$ C. These differences in freezing point denote an osmotic difference of 498 metres water, *i.e.* a pressure of 49,800 grms. per square centimetre. If this work of concentration were carried out by the cells of the tubules, these results would imply that these cells can exert a force six times greater than the absolute force of human muscle (8000 grms. per square centimetre).

Assuming that the whole work of the tubules is confined to the act of concentration, Dreser seeks, moreover, to demonstrate that the glomerular secretion also involves the activity of living cells. Since the blood pressure of 200 mm. Hg = 2·72 metres water, and Δ 1°·0 C. = 122·7 metres water, the highest possible difference between dilute urine and blood, assuming that no concentration had taken place, could only be $\Delta = 0 \cdot °022$ C. Dreser finds, however, that after beer drinking, and

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1892, Bd. xxix. S. 307.

in diabetes insipidus, the urine secreted may have $\Delta = 0°{\cdot}16$ C., *i.e.* a difference between Δ of blood and of urine of ·4° C. Hence he concludes that the production of urine by the glomeruli is also attended with the doing of work, and must therefore be looked upon as a process of secretion. We might, however, still adhere to the theory of glomerular filtration, if we assumed either that the cells of the tubules could absorb water or solids according to the needs of the organism, or that they were able to secrete pure water and so dilute the glomerular transudation.

Heidenhain's criticism of the theory of Ludwig.—The difficulties in the way of accepting Ludwig's hypothesis have led Heidenhain, after a long series of researches on the subject, to reject this theory absolutely, in favour of one very similar to that put forward by Bowman.

Heidenhain sums up his objections to the mechanical theory under the following headings:—

1. The hypothesis that a rise of arterial pressure causes increased transudation through the vessel walls, is not confirmed by our experience in other parts of the body (lymphatics of the limbs, salivary glands).

2. This hypothesis is rendered the more improbable for the kidney, since in this situation the glomerular capillaries are covered by a second layer of epithelium, and we know, from Leber's researches on the cornea, that such a simple epithelial layer can afford great resistance to filtration.

3. If we assume that all the constituents of the urine are filtered off in the glomeruli, the small amount of urea in the blood renders it necessary that in man about 70 kilos. of fluid should be filtered through and reabsorbed, in order that the urea produced in the course of the day may be excreted in the urine,—an amount which is highly improbable.

4. According to the filtration hypothesis, the amount of urine formed must always increase with increased capillary pressure, whereas we find that, on increasing capillary pressure by ligature of the renal vein, the urinary flow is abolished.

5. The hypothesis that the glomerular transudate is concentrated by a process of osmosis or diffusion, on its way through the glomeruli, is rendered impossible by the fact that the osmotic pressure of the urine may be, and generally is, much higher than that of the lymph or blood.

6. The filtration hypothesis does not explain why the amount of urine is increased by the presence of water or crystalloid (*harnfähig*) substances in the blood.

From his own researches on the subject, Heidenhain comes to the following conclusions with regard to the mechanism of secretion:—

1. In the kidney, as in all other glands, the secretion depends on the active intervention of special secretory cells.

2. The first type of these cells is represented by the simple layer of epithelium covering the glomerular loop of capillaries. The office of these cells is to secrete water and such salts of the urine as are found in all other parts of the body in watery solution (*e.g.* sodium chloride).

3. Another system of secretory cells, forming the lining investment of the convoluted tubules and ascending tubule of Henle, secrete the specific constituents of urine (urea, uric acid, etc.). Under some conditions they may at the same time secrete a certain amount of water.

4. The activity of the two kinds of secretory cells is determined—

(*a*) By the amount of water or urinary constituents contained in the blood;

(*b*) By the velocity of the blood-flow through the capillaries of the kidney, inasmuch as on this factor depends the supply of oxygen, and of substances to be excreted, to the cells.

5. The great variability in the constitution of the urine may be explained by differences in the secretory activities of these two types of cell.

The most important part of these conclusions of Heidenhain is a revival of Bowman's theory, that the specific urinary constituents, urea and uric acid, are secreted by the tubules, and that the office of the tubules is secretory rather than absorbent. What evidence have we of the secretory activity of the cells in the tubules?

The great solubility and diffusibility of urea render it impossible to trace this substance on its way through the kidney by micro-chemical means. A better prospect of success would seem to be afforded by the more insoluble uric acid and urates; and both Bowman[1] and v. Wittich[2] have described the presence of uric acid crystals in the cells of the convoluted tubules of birds. Semicrystalline deposits of guanin have been demonstrated with certainty in the cells of the excretory organ of molluscs, but later researches by Adolph Schmidt[3] have shown that the observations of Bowman and v. Wittich must have been due to faulty methods of preparation. Deposited urates were frequently to be seen in the urinary tubules of birds, but never in the cells themselves. In order to throw light upon this point, Heidenhain had recourse to a method, devised by Chrzonzsczewsky,[4] *i.e.* the injection of sodium sulphindigotate (indigo-carmine) into the blood, and the tracing of this coloured substance through the cells of the kidney.

It is found that this substance is excreted in any quantity by two glands only of the body, namely, the liver and the kidney. If 5 c.c. of a saturated watery solution of the sulphindigotate be injected into the veins of a rabbit, within a few minutes the urine becomes a deep blue, and on killing the animal the kidneys are found to be stained blue, the colour being best marked towards the apex of the pyramid. In order to find out in what portion of the secreting substance of the kidney the colouring matter is turned out, the flow of urine must be checked, since otherwise the excreted pigment is at once washed down into the lower parts of the tubules and ureter. To this end Heidenhain[5] divided the spinal cord in the neck. The flow of urine being thus stopped, 5 c.c. of the saturated solution of indigo-carmine is injected into the blood vessels; ten minutes later the animal is killed, and the blood vessels of the kidney washed out with absolute alcohol. By this means the pigment is precipitated *in situ*. On cutting into the kidney, it is at once seen to differ widely in appearance from that of an animal in which the cord was intact. Instead of being diffusely stained, the kidney now is coloured a deep blue in the cortex, the medulla presenting the normal appearance. On examining a section under the microscope, it is seen that the blue colour is due to the deposition of pigment granules in the lumen and in the striated cells lining the convoluted tubules and the ascending

[1] *Loc. cit.* [2] *Arch. f. mikr. Anat.*, Bonn, 1875, Bd. xl. S. 81.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlviii. S. 34.
[4] *Virchow's Archiv*, 1866, Bd. xxxv. S. 158. [5] *Loc. cit.*

limb of Henle's loop, the capsules and the collecting tubules as well as the descending loop of Henle being quite free from pigment.

A very interesting appearance is offered by the kidney, if, previous to the injection, its surface has been cauterised over a small area with silver nitrate, the cord being intact. In the cauterised zones, the secretion of water is stopped, but the excretion of indigo-blue is not affected, so that in these zones the blue colour is confined to the cortex, whereas in the rest of the kidney the coloration is diffuse. Heidenhain concludes from these observations that the excretion of indigo-blue is due to the specific secretory activity of the striated cells lining the convoluted tubules and ascending loop of Henle. Since these cells are the only cells of the kidney which have the power of excreting indigo-carmine, an abnormal constituent of the blood, it is natural to assume that they may also possess the specific function of secreting the urea of normal urine.

These conclusions of Heidenhain's have not, however, passed unchallenged. Various observers have pointed out that, in order to obtain the results described by Heidenhain, it is necessary to repeat exactly all the details of his experiments. If we inject larger doses of the sulphindigotate and kill the animal ten minutes after the injection, it will be found that, in addition to the staining of the striated cells of the convoluted tubules, and the deposition of precipitated pigment in the lumen of these tubules, there is also a slight staining of Bowman's capsule and the glomerular epithelium. It has been suggested[1] that Heidenhain's results might be equally well explained on Ludwig's hypothesis, according to which a dilute solution of the dye would be exuded into Bowman's capsules, and would be concentrated by absorption of fluid on its way through the convoluted tubules. Indigo-carmine is soluble in water and in very weak salt solution, from which it is precipitated on concentration. Moreover, indigo-carmine is liable to reduction in the living tissues with the formation of a colourless product, and these two factors, *i.e.* reduction of the pigment and the extreme dilution of the glomerular exudation, have been held to explain the absence of glomerular staining in Heidenhain's experiment. By increasing the dose injected into the veins and killing the animal soon after the injection, these two factors are minimised and a staining of the capsules is brought about. Sobieranski[2] points out that the staining or deposition of granules in the cells of the convoluted tubules is confined to the parts of these cells bordering on the lumen—a fact which seems to indicate that the pigment has been taken up by these cells from the lumen rather than from the surrounding lymph spaces.

These observations are to a certain extent confirmed by the effects of the injection of carmine. This substance, which has a much more complicated composition than sodium sulphindigotate, enjoys the corresponding advantage of smaller diffusibility, so that it can be more easily traced on its way through the tissues of the body. Moreover, it undergoes no reduction in contact with the living cells. The circulatory disturbance which often accompanies the injection of this substance may be almost

[1] Pautynski, *Virchow's Archiv*, Bd. lxxix. S. 393; Henschen, *Akad. Afhandlung f. medicinska Graden*, Stockholm, 1879 (quoted by Sobieranski); v. Sobieranski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1895, Bd. xxxv. S. 144. (The two first papers are the subject of a critical paper by Grützner, *Arch. f. d. ges. Physiol.*, Bonn, 1881, Bd. xxiv. S. 441.)

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1895, Bd. xxxv. S. 144.

entirely avoided by using a solution of carmine in very weak soda, and carrying out the injection slowly (10 c.c. in five minutes). If we kill the animal thirty to forty minutes after the injection, and wash out the kidney from the renal artery with absolute alcohol, we find the glomeruli stained, the nuclei being red, the glomeruli themselves being of a fainter reddish tinge. The epithelium of the convoluted tubules contains fine granules of pigment towards the inner part of the cells, and here and there deposits of carmine are seen in the straight tubules. Under no circumstances are the pigment granules ever found in the basal parts of the epithelial cells. There can be no doubt that these appearances suggest that the pigment has been taken up by the cells from the lumen rather than that it is in the act of excretion by the cells. In neither of these two experiments do the facts at our command allow us to come to a definite conclusion with regard to their interpretation. In order to decide the relative functions of the glomeruli and convoluted tubules, it would be necessary to separate in some manner the activities of these two parts of the kidney, so as to obtain the action of one or other of them in an isolated form.

Experiments of Nussbaum.—A method for attaining this object was devised by Nussbaum,[1] and promised at first to be of crucial importance for the physiology of urinary secretion. The kidneys of amphibians possess, as Bowman pointed out, a double vascular supply, *i.e.* from the renal artery and from the renal portal vein. From the former vessel are derived the vasa afferentia to the glomeruli, whereas the latter breaks up into capillaries which anastomose round the tubules, in conjunction with the capillary ramifications of the efferent vessels of the glomeruli. Nussbaum imagined, therefore, that the glomerular activities might be altogether excluded by ligature of the renal artery. Carrying out a number of experiments of this description, he obtained results which seemed to decide absolutely in favour of Heidenhain's hypothesis. Thus, after ligature of the renal arteries in frogs, the urinary flow was abolished. A flow of urine might, however, be evoked by the injection of urea into the blood, proving, according to Nussbaum, that the substance was not excreted by the glomeruli but by the tubules, and also that the latter structures could, under the influence of diuretics, secrete part of the water of the urine. In a normal frog the injection of peptone, egg-albumin, or sugar into the blood is followed by the excretion of these substances in the urine. If, however, the renal arteries be previously tied, none of these substances appear in the urine, even when a urinary flow is produced by the injection of urea. Carmine also, which is acknowledged by all observers to be excreted by the glomeruli, does not appear in the urine of the frog, if the renal arteries be ligatured. Nussbaum concluded, therefore, that the excretory apparatus of the kidney consisted of two parts, namely, the glomeruli, which excreted water and salts as well as egg-albumin, peptone, and grape-sugar; and the tubules, which excrete urea and probably uric acid, together with a certain proportion of water.

These experiments are so definite that they would seem to decide the question as to the part played by the various structures of the kidney, were it only possible to place reliance on them. This unfortunately is not the case. A careful repetition of Nussbaum's ex-

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 580.

periments by Adami,[1] working in Heidenhain's laboratory, has shown that in the frog it is impossible to cut off the blood supply to the glomeruli by ligaturing the renal arteries. In fact, after this operation, fully half of the glomeruli may be injected from the aorta, owing to the free anastomoses between the renal artery and the branches of the ovarian arteries and the renal portal vein, and it is difficult to understand how Nussbaum can have obtained the very definite results described by him. These, therefore, in spite of the ingenuity of the methods employed, must be discredited in any discussion concerning the functions of the various parts of the kidney tubule.

Experiments of Ribbert.—A bold attempt to experimentally dissociate the activities of the two portions of the urinary tubule was made by Ribbert,[2] who adopted the method of excising as far as possible the medulla of the kidney, so as to obtain the glomerular secretion after it had passed through only the first convoluted tubules. This operation is only possible in animals such as the rabbit, in which the renal medulla is made up of one Malpighian pyramid. It was carried out in the following way:—One kidney having been exposed from the back, was cut in two by an incision at right angles to the long diameter of the organ, extending into the pelvis. By means of a gouge, as much as possible of the pyramid internal to the boundary zone was removed. The two halves of the kidney were then placed together and secured by sutures, and the other kidney totally excised. Ribbert found that such animals during the next twelve to twenty-four hours secreted a much larger quantity of urine than they had previously done. The urine was more dilute and much lighter in colour than the urine of rabbits under normal conditions. No analyses, however, of the fluid were made. Ribbert interprets these results as confirming Ludwig's hypothesis. But apart from the increased quantity, which does not seem to me to be definitely established by Ribbert's experiments, the production of a more dilute urine would be expected on either hypothesis, whether we assume with Ludwig that the tubules absorb water from the urine, or with Heidenhain that they excrete solid substances into the urine.

Experiments of Bradford.—The very insufficient description of his experiments given by Ribbert might incline us to discredit them altogether, were it not that somewhat analogous results have been obtained by Bradford.[3] This observer found that extirpation of one kidney, combined with excision of a large wedge-shaped piece from the other kidney, might bring about one of two results—

1. If the amount of kidney substance left amounted to one quarter of the weight of the two kidneys, the animals (dogs) lived a considerable time, but suffered from hydruria, *i.e.* the quantity of urine excreted was largely increased, but the excretion of urea remained unchanged, so that the urine was much more dilute than before.

2. If the amount of kidney left was less than one-sixth of the total kidney substance, polyuria was produced, *i.e.* a large increase in the excretion of water as well as of urea. This increased production of urea was due to a rapid wasting of the proteid constituents, and especially of the muscles of the body, so that the animals died in a short time in a

[1] *Journ. Physiol.*, Cambridge and London, 1885, vol. vi. p. 382.
[2] *Virchow's Archiv*, 1883, Bd. xciii. S. 169.
[3] *Proc. Roy. Soc. London*, 1892, vol. li.

state of extreme emaciation. This latter result is difficult to explain, and will be discussed in another section of this volume. The former result (the hydruria) may, however, be analogous to the results of Ribbert's experiments, and may be due to a diminution in the actively absorbing or secretory mechanisms of the kidney, *i.e.* the convoluted tubules. It seems probable that a deficiency in the excretory powers of the organism could be more easily compensated by augmenting the glomerular transudation by means of the blood supply to the glomeruli, than by increasing the work of the cells of the convoluted tubules.

Arguments based upon the reaction of urine.—An objection which has been frequently urged against the filtration hypothesis is that, whereas the blood serum or plasma is in all animals alkaline, the urine, except in those cases where there is a rich supply of alkali in the food, is acid in reaction. It seems difficult to conceive how a process of filtration could effect this change in the reaction of the filtrate. Since all authorities are agreed that the urine undergoes changes in composition on its way through the tubules, it becomes important to find out whether the urine, as it is formed by the glomeruli, is alkaline or acid.

Dreser[1] has sought to determine this question by examining the microchemical reactions in the different parts of the kidney in the frog. As his indicator he used acid fuchsin (rubin S.) This substance is a brilliant red in acid solutions, but is almost colourless in weak alkaline solutions. It is, therefore, a convenient substance to use in order to demonstrate the formation of acid in muscle during tetanus. A strong solution of this dye was injected into the dorsal lymph sac of the frog. An hour or two later the urine that was secreted was of a deep red colour, and was acid in reaction. On examining the kidneys, the dorsal part in which the glomeruli are situated was found to be colourless, but the tubules in the ventral part were filled with red secretion. If the injection were repeated the red coloration extended to the lining cells of the tubules. From his experiments with this and other dyes, Dreser concludes that the production of the acid reaction is effected by the cells of the convoluted tubules, and that the glomerular transudate is alkaline.

This conclusion is borne out by the results of injecting any kind of diuretic. If the glomerular transudate is alkaline, and is also rendered acid in its passage through the tubules, we should expect that the more abundant the glomerular transudate, the shorter would be the time taken in its passage through the tubules, so that the urine pouring into the bladder would tend to approximate in reaction and composition the original glomerular transudate. Such is found to be the case. Whatever means we use to induce profuse diuresis, whether by the injection or administration of drugs such as caffein or theobromin, or the administration of saline diuretics, or the production of hydræmic plethora, we find that the acid reaction of the urine disappears, to be replaced by a neutral or alkaline reaction. We may conclude, with a high degree of probability, that the glomerular part of the urinary secretion is alkaline in reaction, and that the acid reaction of the urine of carnivora or of starving herbivora is due to the changes wrought on the glomerular transudate by the cells of the convoluted tubules. Whether this change is due to the secretion of acid salts, or to the absorption of alkaline salts by the cells of the tubules, we are not in a position to determine.

[1] *Ztschr. f. Biol.*, München, 1885, Bd. xxi. S. 41.

An ingenious attempt has been made by Liebermann[1] to explain the chemical mechanism by which the cells of the tubules effect this change in reaction. This author has described a class of bodies which may be extracted from the mucous membrane of the stomach or from the kidney, and which consist of compounds of lecithin and proteid. These he designates lecith-albumins. These substances are acid in nature, and are capable of combining with alkalies. Liebermann imagines that, as the alkaline salts of the blood plasma pass through the epithelial cells of the kidney, they are split up by these acid insoluble lecith-albumins, which combine with a portion of the bases, so that the remainder of the fluid which reaches the lumen of the tubule contains acid salts or free acid. Of course this process would come to an end as soon as the acid affinities of the lecith-albumins in the cells were satisfied, and in this way one might explain the speedy appearance of an alkaline reaction when large quantities of urine are secreted. Under normal circumstances, however, Liebermann assumes that the carbon dioxide, which is the normal product of tissue metabolism in the kidney, splits up the compound formed in the cells into free lecith-albumin and alkaline carbonates, these latter being then removed by the venous blood stream. As supporting evidence for this hypothesis, Liebermann states that the kidney tissue, like lecith-albumin itself, if treated with soda solution, and then washed repeatedly with water to remove excess of the latter, becomes strongly alkaline. If now the alkaline tissue be suspended in water, through which a stream of CO_2 is passed, and be then again washed thoroughly, it will be found to be strongly acid, having given up all its soda to the carbon dioxide.

Conclusions.—It is evident that the experimental facts at our present disposal do not allow of a definite decision as to the exact manner in which the secretion of urine is effected. It will be convenient, therefore, to summarise the two modes of interpretation, either of which may be applied to the known facts.

According to the Bowman-Heidenhain hypothesis, the secretion of urine is due to the activity of two sets of cells. The flattened epithelial cells covering the glomeruli take up from the blood, circulating through the glomerular capillaries, water and salts, and transfer these substances to the beginning of the urinary tubule. Their activity is chiefly dependent on the activity of the blood flow through the capillaries. But they may be also excited to active secretion by the presence of certain of the urinary constituents in the blood, such as water and salts, or possibly by diuretics, such as caffein. On the other hand, the rodded cells, lining the convoluted tubules and the ascending loop of Henle, secrete specific urinary constituents, such as urea and uric acid, together with a certain amount of water. They also secrete certain abnormal constituents of the blood, such as indigo-carmine. Their activity is chiefly determined by the amount of urea or uric acid in the blood.

If, on the other hand, we accept Ludwig's hypothesis, we must introduce into it certain modifications, necessitated by later inquiries, and assume that in the secretion of urine, as in so many other of the bodily functions, there is a mixture of what we may term physical and physiological processes. It seems probable that in the glomeruli the process is largely if not exclusively physical; that is to say, we have here a transudation of the watery and crystalloid constituents (including urea) of the blood plasma. The extent and nature of this transudation are determined—

1. By the pressure in the glomerular capillaries.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. liv. S. 585.

2. By the velocity of flow through the capillaries.

3. By the permeability of the capillary wall and the glomerular epithelium.

This watery transudate is concentrated and altered on its way through the tubules, in consequence of the absorption of water, and probably of certain of its crystalloid constituents. This absorption must be due to the active intervention of the cells, since the osmotic pressure of the urine is considerably higher than that of the blood pressure. Diuretics may act in two ways. The saline diuretics increase the pressure and velocity of the blood in the glomerular capillaries, not only by increasing the volume of the circulating fluid, but also probably by a direct dilator action on the afferent vessels of the glomeruli. A similar local dilator effect is produced by drugs such as caffein or theobromin; but in these cases the drugs probably exert a paralysing influence on the absorbing mechanism of the kidney, *i.e.* the cells of the convoluted tubules, so that the glomerular transudate may undergo little change on its way to the ureter and bladder.

One of the strongest arguments in favour of this modified Ludwig hypothesis is the fact that the more we augment the flow of urine, whether by caffein, saline diuretics, or production of hydræmic plethora, the more nearly does its osmotic pressure, saline constitution, and reaction approximate that of the blood plasma. It would seem that in the glomeruli we have an apparatus which, like the capillaries of the abdominal viscera but in a still higher degree, reacts to changes in the intracapillary pressure, and so serves to regulate accurately the amount of fluid circulating in the blood vessels.

Whether we look upon the cells of the convoluted tubules as secretory or absorptive in function, we have at present no evidence that the cellular covering of the glomeruli acts otherwise than passively in the production of the glomerular part of the secretion. It must be remembered, however, that under certain circumstances, as after ingestion of large quantities of fluid, the osmotic pressure of the urine may fall below that of the blood plasma. Dreser[1] interprets this as pointing to an activity of the glomerular epithelium. I have shown above that it may equally well be explained by assuming an absorption of salts by the water-logged tubule cells, or an active excretion of water by these cells. I may mention here that I. Munk and Senator,[2] as a result of researches carried out for the most part on the excised kidney, have come to a conclusion analogous to that just stated, namely, that in the production of urine we have a co-operation of physical and physiological factors. According to these authors, water and part of the urinary salts (especially NaCl) are transuded through the glomeruli in direct consequence of the blood pressure, *i.e.* by a process of filtration, although the rapidity of the blood flow is at least of equal importance with its pressure. The specific urinary constituents—urea, uric acid, hippuric acid, etc., together with another portion of the urinary salts (NaCl, sulphates and phosphates) —are secreted by the active intervention of the cells of the tubules, especially the convoluted tubules. These substances are secreted in a dissolved condition, and must, therefore, take a certain amount of water with them.

The influence of the nervous system on the secretion of urine. —The discovery by Berkeley[3] of a distribution of nerve-endings to the

[1] *Loc. cit.* [2] *Virchow's Archiv*, 1888, Bd. cxiv. S. 1. [3] *Loc. cit.*

tubules of the kidney suggests that in this organ, as in the salivary glands, the secretion of urine may be under the direct control of the central nervous system, apart from any influence that this system may have on the renal circulation. We have already seen that the urinary secretion is extremely susceptible to variations in the pressure and velocity of the blood in the renal vessels, and also that these latter are under the direct control of the nervous system by means of vaso-dilator and vaso-constrictor nerve fibres.

Various authorities have described experiments which should demonstrate the existence of secreto-motor nerves to the kidney. Thus, in 1835, Claude Bernard[1] showed that in some cases, where puncture of the medulla was carried out with the view of producing diabetes, the result was an increased flow of urine, containing no sugar, *i.e.* diabetes insipidus. These experiments were repeated in much greater detail by Eckhard,[2] who showed that, in the rabbit, polyuria might be caused, not only by a puncture of the medulla, but also by chemical or mechanical stimulation of the neighbouring portion of the superior vermis of the cerebellum, especially if, previously to the operation, the nerves going to the liver had been divided. Moreover, it is a familiar fact to clinicians, that injuries to the head, epileptic attacks, and especially lesions in the neighbourhood of the medulla, may bring about a condition of diabetes insipidus.

We know already that division of one splanchnic nerve will cause an increased secretion of urine in the kidney of the same side, and it is natural to imagine that the mechanism of the increased urinary section after the *piqûre* is of the same nature. Eckhard pointed out, however, that the course of events is different in the two cases. After division of one splanchnic, the flow of urine is almost immediately somewhat increased, and this moderate increase lasts a considerable time (three to four hours at least). The first result of puncture of the medulla is a cessation of the urinary flow. This is followed shortly by an increase much greater than is caused by section of the splanchnic, but only lasting one to two hours. Moreover, the effect of the diabetic puncture is observable even after section of the splanchnics, as well as of all the nerves which may possibly send branches to the kidney. Eckhard concludes, therefore, that the effect must be due to one of two causes: either an increased general blood pressure, in consequence of the stimulus caused by the puncture, or the excitation of nerve fibres which run in the walls of the renal artery itself. The first explanation must be rejected, since direct measurement of the blood pressure does not show any definite rise in consequence of the puncture. We must therefore accept the second explanation as the correct one. Eckhard regards these nerve fibres as secreto-motor, and believes that the urinary secretion is under the control of a nerve centre, situated most probably in the medulla. He bases this hypothesis on the facts that section of the cord below the medulla stops the flow of urine, and that stimulation of the cut cord does not bring back the flow, in spite of the rise of blood pressure which is induced. We know now, however, that the negative result of stimulating the cut cord is due to the constriction of the renal vessels, which occurs together with those of other parts of the body, so

[1] "Leçons de physiol.," 1835, tome i. p. 339.

[2] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1869, Bd. iv. S. 1–32 and 153–193; 1870, Bd. v. S. 147–178; 1872, Bd. vi. S. 1–18 and 51–94.

that the increased general blood pressure is powerless to send more blood through or to raise the pressure in the renal capillaries.

The facts can be equally well explained if we assume that these hidden nerve fibres are vaso-dilator in function—an assumption which would be in accord with the numerous other facts we have learnt with regard to the regulation of the urinary flow by the central nervous system. We may conclude, therefore, that the existence of secretory nerves to the kidney is not proved, the subjection of the renal secretion to nervous influences being effected exclusively through the intermediation of the vascular nerves.

As an additional argument against the dependence of renal secretion on the nervous system, Heidenhain quotes a number of experiments made by Bidder[1] on frogs, in which the secretion of urine continued normally, although in some animals the whole spinal cord, in others the whole nervous system, with the exception of the medulla, had been destroyed.

[1] *Arch. f. Anat. u. Physiol.*, Leipzig, 1844, S. 376.

THE MECHANISM OF THE SECRETION OF MILK.

By E. A. Schafer.

Contents :—General Considerations, p. 662—Influence of the Nervous System, p. 663—Action of Pilocarpine and Atropine, p. 664—Influence of Diet, p. 664—Place of Formation of the Organic Constituents, p. 665—Manner in which the Secreted Materials pass out of the Cells, p. 665—Mechanism of the Discharge of Milk, p. 667.

The composition of milk has been dealt with in a previous article (pp. 125 to 140). Here it may therefore be simply noted, with regard to its organic constituents, that these are remarkable in being peculiar to the milk, not occurring in any of the other secretions or tissues of the body (cf. however, footnote 1, p. 665), nor in foods which have not been prepared from milk. The mammary gland-cells, therefore, unquestionably form the products of secretion themselves from materials derived through the lymph from the blood, and cannot be regarded, except as concerns some of the inorganic substances, as acting merely as filtering agents for allowing the passage of materials in solution from the blood. And even with regard to the inorganic substances,[1] the proportion of these is so different from that in which they occur in the blood and lymph, that no filtration hypothesis appears in any way tenable even for these. The gland-cells are further peculiar in that they only, as a rule, function actively for a certain period after parturition, being at all other times entirely inactive, although capable occasionally—it is said even in the male—of being excited to activity by stimulation of the nipple by a sucking action, such as that performed by an infant. Prior to, and during such periods of activity, the whole gland becomes greatly enlarged, both by an increase in size of existing alveoli, and also, perhaps, by a sprouting out of new alveoli. The cells lining the alveoli become enlarged, and probably also multiply, for they are said to show evidence of karyokinesis.

The alveolar cells begin to accumulate within them granules, partly of a proteid, partly of a fatty nature (although the latter may more fitly be described as globules), and the alveoli get filled, before there is any call for the pouring out of the secretion, with a clear fluid (coagulating to a finely granular material in pieces of the gland thrown into alcohol), which contains a few fatty globules of different sizes, and here and there cells filled with granules, staining with osmic acid, and apparently identical with the *colostrum corpuscles* which are found in the milk of

[1] Bunge has shown that, with the exception of iron, the inorganic substances of milk occur in nearly the same proportion as in the ash of new-born animals ("Text-Book," Wooldridge's translation, p. 107).

the first two or three days after parturition, and which are sometimes even to be detected during full lactation. These colostrum corpuscles are seen to be amœboid when examined on the warm stage, and are, there is little doubt, leucocytes which have wandered out from the interstitial connective tissue of the gland into the lumen of the alveoli. Some have regarded them as detached epithelial cells, and look upon their presence in the alveoli and in the milk as evidence of the normal occurrence of such detachment during active secretion (see p. 666); but it must be admitted that they have neither the appearance of epithelial cells, nor do the latter tend to exhibit any such amœboid movement as is shown by the colostrum corpuscles. These corpuscles, in fact, seem to be rather analogous to the *salivary corpuscles* (see p. 344), and to be similarly derived from emigrated leucocytes.

During the period of lactation the alveoli secrete milk, not only whilst the gland is being drawn by the process of sucking or milking, but in the intervals of such processes, so that the milk accumulates both in the alveoli and in the ducts. The latter are provided with (in some animals very considerable) dilatations, which serve as reservoirs for the accumulated secretion, and it is mainly this accumulated milk which is poured out during the milking. No doubt fresh milk becomes secreted to take the place of that which is drawn away; and as a concomitant to this fresh secretion, there is a considerable flush of blood to the gland. It has been calculated that the udders of a cow could not contain all the milk which is sometimes drawn at one milking, so that secretion must be proceeding at the same time. Moreover, the later drawn portions of milk contain more solids in proportion than those first drawn.[1] Lehmann[2] injected sulphindigotate of soda solution into a vein of a milch goat, and at once had the animal milked. No blue appeared in the milk until the udders were almost completely drawn, when there was a slight tinge. On milking the animal again, after the lapse of an hour or an hour and a half, the milk which had collected in the udder was completely blue.

Influence of the nervous system on the secretion of milk.—Although it is a matter of common experience that the quantity and quality of the milk is in women materially influenced by the condition of the nervous system, the results of experiments upon animals have furnished evidence on this subject which is either entirely negative, or at most of a somewhat conflicting nature. Eckhard,[3] who was the first to attempt to obtain such evidence, found no marked difference in the milk either in quantity or quality from the udder of a goat, the nerves (branches of external spermatic) passing to which had been cut, as compared with the milk drawn from the other side, the nerves of which were intact. His observations have been repeated by others,[4] with contradictory results, some having obtained an increase of secretion on cutting the nerves, others a diminution. But even if an increase is obtained, it has not been determined whether this is due to the alteration in the vascular supply to the gland rather than to a direct effect upon the gland-cells, such as is obtained in the case of the

[1] For references, see Heidenhain, Hermann's "Handbuch," Bd. iv.

[2] *Die landwirthsch. Versucht*, 1887, Bd. xxiii. S. 473.

[3] *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1855.

[4] Röhrig, quoted by Heidenhain (Hermann's "Handbuch," Bd. iv.); de Sinéty, *Gaz. méd. de Paris*, 1879, p. 593; Valentowicz, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1888, Bd. ii. S. 71; Mironow, *Arch. de sc. biol.*, St. Pétersbourg, 1895, tome iii. p. 453.

(paralytic) secretion of saliva after section of the chorda tympani. Likewise, the effects which have been got by stimulating the cut nerves, and which have been usually in the direction of diminishing the quantity of the secretion, may well be ascribed to vasomotor changes rather than to direct nervous influence. All that can be said, therefore, on this question is to repeat the statement, that the experimental evidence of such an influence is still lacking, however probable its existence may be from the everyday experience of changes produced in the milk of nursing women, as the result of emotional conditions.

Action of pilocarpine and atropine.—The drug which has the most marked effect in increasing most of the secretions of the body, namely, pilocarpine, is stated to have little or no effect upon the secretion of milk.[1] On the other hand, atropine is well known to be constantly employed for nursing women, in whom, for one reason or another, it is desired to dry up the secretion. Short, however, of stopping the secretion altogether, atropine, given in smaller doses, is found, whilst diminishing the amount of fluid secreted, to cause the secretion of a more concentrated milk.[2]

Influence of diet.—The quantity and quality of the food is well recognised as having an important influence on the quantity and quality of the milk. The most abundant and richest milk is yielded when the diet is liberal, and, in the case of carnivora (bitch) certainly, but less certainly in the case of herbivora (cow), when it includes a larger proportion than usual of proteid material. And it is not so much the albuminous constituents of the milk (casein and lact-albumin) which are increased, but especially the proportion of fat.[3] This indeed has been held to be one of the most cogent arguments in favour of the view contended for by Voit, that animal fat is formed mainly from proteids.[4] An increase of fat in the food, without a simultaneous increase of proteid, does not cause an increased secretion of fat in the milk.[5] Not only the amount of proteids and fat, but also the amount of sugar, is increased as the result of giving proteid-rich food.[6] Alcohol, given to goats, has also been found to increase the fat of milk.[7]

It does not, of course, follow that because an excess of a particular organic principle in the food produces an increase of certain constituents of the milk, that these constituents are directly produced from such material, for the effect may be produced indirectly by the functions of the gland-cells becoming modified, according to the nature of the pabulum they are receiving. Looked

[1] Hammarbacher (goat), *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiii. S. 228; Cornevin (*Compt. rend. Soc. de biol.*, Paris, 1891, p. 628) found that in the cow the amount of milk yielded was not influenced by the daily injection of 0·25 grm. pilocarpine. See also Mironow, *loc. cit.*

[2] Hammarbacher, *loc. cit.*

[3] The evidence for this is given by Heidenhain (Hermann's "Handbuch," 1882, Bd. v.), where also all the most important references on the influence of diet up to that date will be found. The following may also be cited—W. Kirchner, *Milchzeitung*, 1891, Bd. xx.; C. Schneider, "Einfluss versch. Fütterung auf d. Zusammensetz. der Milch," Diss., Leipzig, 1893.

[4] See article on "Metabolism."

[5] Ssubotin, *Virchow's Archiv*, 1866, Bd. xxxvi.; *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, S. 337; Kemmerich, *ibid.*, S. 467; Kuhn, *Journ. f. Landwirthsch.*, 1876, S. 381; Weiske, *ibid.*, 1878, S. 447; Cf. also Juretschke, "Einfluss versch. Oelkuchensorten auf dem Fettgehalt der Milch," Diss., Leipzig, 1893.

[6] I. Munk, *Arch. f. wissensch. u. prakt. Thierh.*, Berlin, 1881, Bd. vii. S. 91.

[7] Stumpf, *Deutsches Arch. f. klin. Med.*, Leipzig, 1882, Bd. xxx. S. 201.

at in this light, certain substances may be said to stimulate the cells of the glands to increased activity in all directions, tending to the production of a larger quantity of milk rich in all kinds of solid constituents; whilst other substances may be looked upon as stimulating the cells in a special manner, tending to the increased production of certain only of the constituents of the milk.

Place of formation of the organic constituents.—As already noticed, the fact that the chief organic constituents of the milk are peculiar to the secretion, and do not occur as such in the blood or lymph, may be regarded as sufficient evidence of their being formed in the gland itself.[1] The casein is in all probability produced by a molecular change in the composition of the serum albumin or globulin, which is supplied to the cells from the blood or lymph. The fat may be formed by the cells of the gland from proteid, or possibly even from carbohydrate materials furnished by the blood; or it may be taken up directly from fat which has been formed elsewhere, and which is always present in a certain small amount in blood and lymph. For while, on the one hand, there exists no clear evidence to show that the mammary gland can itself manufacture fat, it is extremely probable that, in common with most if not all other cells in the body, the cells of this gland do possess such a faculty. With regard to the characteristic sugar of the secretion, and which, being characteristic, must be produced by the gland itself, there is some evidence to show that this is formed from dextrose, which is itself manufactured elsewhere than in the gland. That this is so would appear from the following experiment by Paul Bert.[2] Bert removed the mammary glands from goats, then allowed them to become pregnant. After parturition, the urine, during three days, contained a substance which reduced cupric oxide and appeared to be dextrose. This was not present before parturition, nor was it found in normal animals either before or after parturition; it was therefore presumably formed in the organism in larger amount than usual for the purpose of becoming converted into lactose in the mammary gland.

In view of the fact that lactose is frequently found in the urine in women, and in mammals generally, immediately before and after parturition,[3] this experiment of Bert seems to need repetition, especially since he appears not to have isolated or carefully examined the reducing substance which he detected. The lactose found in the urine after parturition has generally been supposed to be re-absorbed from the secretion which has formed in the alveoli and ducts of the mammary glands.

Thierfelder[4] has pointed out that both the casein and sugar of milk could be derived from the nucleo-proteids or glyco-proteids of the gland-cells by a process of splitting. In support of this view, a formation of lactose is said to occur on keeping portions of minced fresh mammary gland in normal saline solution at the temperature of the body; the lactose being preceded by a colloid carbohydrate, identical, according to Landwehr,[5] with his "animal gum." This, however, does not affect the question of the ultimate sources of origin of these

[1] A small quantity of casein is said to occur in the secretion of the sebaceous glands (Neumeister, "Lehrbuch," Aufl. ii. S. 496). This is of interest in connection with the fact that the mammary glands have been regarded as representing enlarged and modified sebaceous glands.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1884, tome xcviii. No. 13.

[3] Hofmeister, *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. i. S. 101.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1883, Bd. xxxii. S. 619.

[5] *Ibid.*, 1887, Bd. xl. S. 21.

constituents, which are probably, as already stated, the proteids and carbohydrates derived from the food.

As to the manner in which the secreted materials of the milk pass out of the secretory cells.—If we put aside, as resting upon no solid basis of fact, the suggestion of Stricker, which was taken up more seriously by Rauber,[1] that the organic materials of the milk are carried into the alveoli by emigrated leucocytes, which there break down and set free their proteid, fatty, and other constituents, we find ourselves face to face with three possible methods by which the secreted materials which are formed and accumulate within the gland-cells may pass into the lumen of the alveoli. The three methods are as follows:—

1. The cells may, as in the case of the sebaceous glands, bodily break loose, and, becoming detached and disintegrated, set free their contents within the alveoli.

2. A part only of each cell, namely, the free end, may break loose, become detached, and disintegrate.

3. The cells may extrude their secreted materials into the alveoli, much as in the case of other secretions, without undergoing any histological disintegration.

Of these three views, the first has found support mainly on the ground of the analogy with what happens in the case of the sebaceous glands of the skin (with which the mammary glands might be looked upon as in a certain sense homologous), in which such a complete disintegration of the whole cell occurs, its place being supplied by another cell, which is produced by cell-division. Moreover, the colostrum corpuscles have been regarded as examples of such detached cells filled with secretion, which have not become disintegrated. Those corpuscles, however, as we have seen, are rather to be looked upon as of the nature of leucocytes than as epithelial cells; nor do we find such evidence of cell multiplication in the mammary gland as would be at all sufficient to account for the very large number of cells which would have to become detached in order to furnish the organic constituents of the secretion. Heidenhain[2] has calculated that the gland-cells would have to be totally renewed five times in the course of every twenty-four hours, in order to yield the solids of the milk. The second view may be looked upon as, in a sense, a modification of the first one. It was due originally to Langer, and has been ably advocated by Partsch and Heidenhain.[3] According to this view, the secretion products which are formed in the gland become gradually accumulated within the free ends of the cells, which in the meanwhile lengthen out, and in place of being flat or cubical become columnar and project into the lumen of the alveolus. The enlarged free end is then supposed to burst or to become detached and disintegrated, and thus to set free the accumulated products, while the fixed ends of the cells (with the nuclei) are supposed to remain, ready to again go through a similar process.

The evidence adduced in favour of this view is chiefly of a histological nature. It is the case that in some alveoli of glands in full secretion, the cells are occasionally seen projecting somewhat prominently into the lumen; and it is certainly the case also that the cells, and perhaps especially such prominent parts, contain fatty globules, similar in

[1] "Ueber den Ursprung der Milch," Leipzig, 1879.

[2] *Loc. cit.*

[3] *Vide* Heidenhain, *op. cit.*

appearance to those of the milk. Nevertheless, it must be admitted that such appearances, although they may occasionally be seen, are decidedly rare. It is so much more common to see, even in the most actively secreting glands, the alveolar cells uniformly flattened, or at most very slightly projecting, that I should not hesitate to say that the columnar appearance, which has been described and figured by Heidenhain, is quite exceptional, and is in all probability due to the alveoli in which it occurs being collapsed. Every histologist is aware of the extreme differences in shape which are produced in epithelial cells by alterations in the conditions of the surface which they cover. Thus, even the extremely flattened epithelial cells which line the blood vessels may, when examined in sections of vessels which have been hardened in a contracted and collapsed condition, project like columnar epithelium cells into the lumen of the vessel. And the differences in height of the cells lining the alveoli of the mammary glands may very well be similarly produced. This is indeed rendered probable from the observation of Heidenhain,[1] that in different lobules of a gland the cells vary in height, but in the same lobule they have the same height; and by the additional observation of the same observer, (*a*) that in a bitch which was suckling seven vigorous puppies the cells were very high; (*b*) that in another well-fed milch bitch, which was not sucked for forty-eight hours, they were remarkably low; for the alveoli in these cases would be flaccid and tense respectively.

The histological evidence in favour of this view must therefore be admitted to be extremely weak, nor, except perhaps in the case of the unicellular glands of some invertebrates, and the similar unicellular secreting structures which form the mucus-secreting goblet-cells of vertebrates, is there any analogous instance of the extrusion of a secretion by the breaking down of part of the gland-cells. Moreover, the argument which was used by Heidenhain against the first view, that it would involve the renewal of the substance of the epithelial cells of the mammary gland five times in twenty-four hours, will apply with slight modification equally to the second, and adds a further considerable difficulty to its acceptance.

The third view, on the other hand, has the analogy of nearly all the other secretory structures to support it. It involves no necessity for assuming such an enormous building up and breaking down of protoplasm as is required for the other two; and although we must admit that the present state of our knowledge does not permit us to understand how and why it is that certain substances are formed in these cells, and pass from them into the lumen of the alveolus, the same admission must be made for all other secretions. Nor is the fact that the fat of the milk is extruded from the cells in an undissolved condition any obstacle to the acceptance of the view in question, since it is probable that the granules which are found in many other secretory cells (*e.g.* those of the salivary glands), and which are passed into the lumen of the alveolus, are extruded as granules, and are first dissolved in the secretion outside the cells.[2]

[1] *Loc. cit.*

[2] The microscopical changes in the cells of the mammary gland during secretion have been recently made the subject of study by Steinhaus (*Arch. f. Physiol.*, Leipzig, 1892, Suppl., S. 54) and Szabo (*ibid.*, 1896, S. 32). The former finds evidence of frequent mitotic division of the cell nuclei (without subsequent division of the cells), and of transforma-

The discharge of milk.—The discharge of milk from the ducts, which is produced by the action of sucking or milking, is partly the result of direct mechanical pressure upon the gland, and especially upon the milk reservoirs of the larger ducts, partly due to a contraction of the plain muscular tissue which accompanies these ducts, and which appears to be set in action by mechanical stimulation of the nipple. The plain muscular tissue occurs also in the nipple itself in some abundance, and by its contraction causes a kind of erection and increased prominence of the nipple. This probably serves the purpose of keeping open the mouths of the gland-ducts which open upon the rounded apex of the nipple, thus allowing of the free outflow of the secretion.[1] The flow is also in all probability assisted by a *vis a tergo*, derived from the swelling of the whole gland by the reflex dilatation of its arterioles, and consequent increase of capillary pressure, and of lymph exudation; and, to a slight degree also, by newly secreted milk, which begins to be formed by the gland-cells, in response either to this increased supply of blood and lymph, or to reflex secretory influences passing directly to the gland-cells.[2]

tion of the nuclear substance into fat. The latter could find no mitoses during lactation, although he found two or three nuclei in each cell. He also describes the accumulation in the cells of albuminous granules which undergo peculiar changes of form, and are ultimately extruded into the alveoli and there dissolved. These observations require corroboration.

[1] Cf. Hellier "On Nipple Reflex," *Brit. Med. Journ.*, London, Nov. 7, 1896.

[2] Cf., however, what has been already said on this subject on p. 663.

SECRETION AND ABSORPTION BY THE SKIN.

By E. Waymouth Reid.

Contents :—Chemical Nature of Skin Secretions, p. 669—The Secretion of Sweat, p. 676—Electro-Motive Phenomena in Skin Glands, p. 681—Absorption by the Skin of Man, p. 685—Of lower Mammals, p. 688—Of the Frog, p. 690.

Skin Secretions.

Comparative.—The secretions of the skin in vertebrates fall readily into two main classes—(*a*) Those in which a watery solution is elaborated by the gland-cells, and (*b*) those in which products of metamorphosis or degeneration of the gland-cells themselves form the secretion.

As types of the former class may be instanced the sweat of mammals, and the slime of fish and many amphibians; of the latter, the secretion of the various modifications of sebaceous glands in mammals; of the uropygial gland of many birds; and the fibre secretions of the skins of certain fish (*Myxine*, *Anguilla*, etc.).

Such secretions are put to a variety of uses in the vertebrate series. Of the first class, the sweat of the mammal is at once an excretion and a means of regulating body temperature by evaporation, while the slime of the frog or fish is protective in function. Of the second class, the greasiness of the sebum of the mammal, or secretion of the tail gland of the bird, protects skin, hair, or plumage from imbibition of water; the secretion of the Meibomian glands of the eyelids prevents overflow of tears; the viscosity of the ear wax interferes with the entrance of foreign bodies into the auditory canal; while, in special cases, volatile substances of good or evil odour, contained in the secretion, may serve the purposes of sexual attraction or protection from enemies.

In hairy mammals, it is only in certain cases, or on certain parts of the body, that sweating is observed. Rabbits, rats, and mice are not known to sweat at all, the dog sweats but little, the cat only on the hairless pads of the feet; while on the other hand the horse sweats profusely on all parts. The snouts of pigs and oxen contain glands similar to sweat-glands, the secretion of which keeps the part moist.

Instances of glands used for purposes of sexual attraction are—the glands of the suborbital pit of many ruminants and some hogs, the cheek gland of the elephant, the pectoral glands of certain tropical bats, the flank glands of shrews, the sacral gland of the peccary, the groin glands of antelopes, the preputial glands of the beaver and musk-deer, the anal glands of the hare, marsupials, armadillos, two-toed sloth, otter, hyænas, and civets, and the glands at the base of the tail of shrews and the fox. The anal glands of the skunk are used for protection.

The hoof gland of most bisulcate ungulates, opening in the cleft between the two divisions of the hoof, is probably of use in protecting the horny

matter from imbibition of water; in the one-horned rhinoceros a gland opens on the posterior aspect of each foot.

The function of the curious gland at the back of the thigh of male monotremes, supplying its secretion by a long duct to the hollow horny spur on the heel (so like in arrangement to the poison gland and fang of a snake) is not known with certainty.

There are glands in the skin of the male of the kangaroo, *Halmaturus rufus*, which secrete a red substance adhering to the hair, while the maxillary glands of the female dwarf antelope, *Cephalolophus pygmæus*, secrete a blue substance reddened by acid.[1]

Chemical Nature of Skin Secretions.

(*a*) **Watery secretions.**—Naturally the composition of the sweat of man and mammals has received more attention than that of other skin secretions.

Since the quantity of sweat secreted is dependent upon so many conditions, it is of little value to quote the numbers obtained by different observers, apart from a statement of the special conditions under which the observations took place.

There are several methods of collecting the sweat of the whole body, or of special parts. Evaporation may be hindered by enclosing a part, such as the forearm and hand, in a rubber bag, and the sweat collected in a bottle tied into the lower end of the bag.[2] The subject may sit in a Pettenkofer and Voit's respiration chamber (but breathe through tubes to the exterior), and the water given off by the skin be calculated from the readings of hygrometers in the ingoing and outgoing currents of air.[3] Or the secretion of the skin may be stimulated by raising the temperature of the surrounding air, while the whole body, with the exception of the head, is enclosed in a convenient receptacle.[4] By the hot-air method, Argutinsky[5] collected a quarter of a litre of sweat in half an hour, at a temperature raised during the experiment from 27° to 41° C. Schierbeck,[6] by the hygrometric method, calculated that in his own case, when clothed and at rest, the air in contact with the skin being at the normal temperature within clothing (32° C.), 2 or 3 litres of sweat were given off in twenty-four hours. No calculations of the total secretion of sweat can be made from local estimates, because the richness of various districts of the skin in sweat-glands is very different.

In the body at rest the sweat is evaporated as fast as it is formed, and it is only under conditions exciting the glands to increased action, that the fluid collects upon the surface.

In the resting condition of the body the temperature of the surrounding air must be raised to about 33° C. before the stimulus to increased activity of the sweat-glands is evoked.[7]

The following table is of interest as indicating that, at the time of

[1] Weber, *Arch. f. mikr. Anat.*, Bonn, 1888, Bd. xxxi. S. 499. For further information on such glands, see Owen, "Comparative Anatomy and Physiology of Vertebrata," London, 1868, vol. iii. p. 632; Leydig, *Ztschr. f. wissensch. Zool.*, Leipzig, 1850, Bd. ii. S. 1 (anal glands).

[2] Anselmino, Wagner's "Handwörterbuch d. Physiol.," art. "Haut."

[3] Schierbeck, *Arch. f. Physiol.*, Leipzig, 1893, S. 116.

[4] Favre, *Compt. rend. Acad. d. sc.*, Paris, 1852, tome xxxv. p. 721.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 594.

[6] *Loc. cit.*

[7] Schierbeck, *loc. cit.*

the breaking out of sweat, the excretion of carbon dioxide by the skin is also suddenly increased, a fact probably related to the increased activity of the gland-cells :—

Excretion of Water and Carbon Dioxide by the Skin at various Temperatures of Surrounding Air.

(Separate experiments on same individual, naked, in a Pettenkofer and Voit's chamber).[1]

Temperature of Chamber.	Water Excretion (Grms. per Hour).	Water Excretion (Grms. per Twenty-four Hours).	Carbon Dioxide (Grms. per Hour).	Carbon Dioxide (Grms. per Twenty-four Hours).
29°·8 C.	22·2	532·8	·37	8·9
30°·4 ,,	27·8	667·2	·40	9·6
31°·5 ,,	71·9	1725·6	·37	8·9
31°·9 ,,	50·3	1207·2	·35	8·4
32°·8 ,,	73·4	1761·6	·35	8·4
33°·8 ,,	82·6	1982·4	·87	20·9
35°·4 ,,	106·8	2563·2	1·04	25·0
35°·7 ,,	107·0	2568·0	·9	21·6
38°·4 ,,	158·8	3811·2	1·23	29·5

The sweat of man is a colourless, opalescent liquid of salt taste, poorest in solids of all the secretions, though richest in salts in relation to organic solids.

Sweat is acid in reaction, even when collected from the palm of the hand, where there is no danger of admixture of sebaceous secretion.[2] This acidity is probably due to volatile fatty acids, which may be subsequently driven off, leaving an alkaline reaction at the surface of the skin.[3] In profuse sweating the acidity may give way to neutrality, followed later by alkalinity.[4]

In 1000 parts.	Favre.	Schottin.	Funke.
Water	995·573	977·40	988·40
Solids	4·427	22·60	11·60
Epithelium . . .	...	4·20	2·49
Fat	·013	...	...
Lactates	·317	...	...
Sudorates	1·562	...	...
Extractives . . .	·005	11·30	...
Urea	·044	...	1·55
Sodic chloride . . .	2·230	3·60	...
Potassic chloride . .	·024	...	...
Sodic phosphate . .	Traces	1·31	...
Alkaline sulphates . .	·011	·39	...
Earthy phosphates . .	Traces	...	...
Total salts	...	7·00	4·36

NOTE TO TABLE.—The sudoric or hidrotic acid of Favre has not been found by any subsequent observers. He gives the empirical formula, $C_{10}H_{16}H_2O_{13}$. Lactic acid also has not been found by any other observer.[5]

[1] Schierbeck, *loc. cit.*

[2] François-Franck, "Dict. encycl. d. sc. méd.," Paris, 1884, Sér. 3, tome xiii. p. 51, Art. "Sueur."

[3] Tourton, "Thèse de Lyon," 1879, No. 24, Sér. 1.

[4] Favre, *loc. cit.* ; Trümpy and Luchsinger, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii. S. 494.

[5] For analysis of sweat of a rheumatic patient, see Harnack, *Fortschr. d. Med.*, Berlin, 1893, S. 91 ; also Hermann, *Jahresb. ü. d. Fortschr. d. Anat. u. Physiol.*, Leipzig, 1895, Bd. ii. S. 226.

The specific gravity of human sweat is 1003 to 1006.

The table on p. 671, from Beaunis,[1] gives the composition of sweat according to Favre,[2] Schottin,[3] and Funke : [4] —

Relatively to the chlorides, the sulphates and phosphates of sweat are less abundant than in urine. The following table is from Kast : [5] —

	Chlorides.	Phosphates.	Sulphates.
Sweat . .	1	·0015	·009
Urine . .	1	·132	·397

There is no doubt that urea is present in the sweat of man; the variations in estimates of the amount by different observers being probably caused by differences in the lapse of time between collection and estimation, and consequent variations in the amount of transformation into ammonium carbonate.

In two lots of sweat collected by the hot-air method, Argutinsky[6] found that ·363 grm. urea was present in 225 c.c. of sweat collected in half an hour, and ·410 grm. urea in another sample of 330 c.c. collected in three-quarters of an hour.

Of the total nitrogen excreted by the skin in one case, 68·5 per cent. was present in urea, and 31·5 per cent. in ammonia; while in the other the numbers given are 74·9 per cent. of total nitrogen in urea, and 25·1 per cent. in ammonia.

The same observer, by taking severe walking exercise in a special suit of clothes, which was extracted at the end of the period of work, and the extract analysed by the Kjeldahl method, obtained results as follows:—

Work.	Mgrms. of Nitrogen excreted by the Skin.
20 to 22 kilometres in seven hours (July) . . .	704·4
18 to 20 „ with ascent of 1300 metres (August) .	753·5
„ „ „ 1600 metres (October)	219·3

The nitrogen excreted by the skin may amount to 4·7 per cent. of that by the urine, and hence may have to be taken into account in some experiments on nitrogenous metabolism.

In uræmic conditions, the excretion of urea by the skin is greatly increased, so much so, in some cases, that crystals of urea have been found on the skin.[7]

According to Capranica,[8] creatinine to the extent of ·04 per cent. is present in human sweat. The small amounts of fatty acids are made up of formic, acetic, butyric, propionic, and caproic acids. Ethereal sulphates of phenyl and skatoxyl are present in small amount, the proportion of ethereal to inorganic sulphates being, according to Kast,[9]

[1] "Nouveaux eléments de physiologie humaine," Paris, 1888, 3rd edition, tome ii. p. 190.

[2] *Loc. cit.*

[3] "De Sudore," Diss., Leipzig, 1851.

[4] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1858, Bd. iv. S. 36.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1887, Bd. xi. S. 501.

[6] *Loc. cit.*

[7] Schottin, *loc. cit.*

[8] *Arch. ital. de biol.*, Turin, 1882, tome ii.

[9] *Loc. cit.*

1 to 12. Indigo is sometimes developed in sweat,[1] though whether from indoxyl secreted, or as the result of the growth of chromogenic micro-organisms, is not certain.

The sweat of the horse has been studied by Leclerc [2] and Fred Smith.[3] This secretion normally contains proteids, a fact which may partly account for the debilitating effects of profuse sweating in horses.

Percentage Composition of Sweat of Horse (Fred Smith).

Alkaline, sp. gr. 1020 ; Water, 94·3776 ; Organic solids, ·5288 ; Ash, 5·0936.

Serum albumin	·1049
Serum globulin	·3273
Fat	·0020
Chlorine	·3300
Lime	·0940
Magnesia	·2195
Phosphoric acid	Trace
Sulphuric acid	Trace
Soda	·8265
Potash	1·2135

Both Leclerc and Smith found urea in the sweat of the horse.

The sweat of the hippopotamus contains a reddish-brown pigment not yet identified.[4]

Buisine [5] has investigated the constituents of that part of the "sweat" of sheep which is soluble in water. He found potash soaps of the fatty acids from acetic to capric ; urea and ammonium carbonate ; potash salts of malic, glycolic, pyrotartaric, oxalic, succinic, lactic, hippuric, benzoic, and uric acids ; phenylsulphate of potassium, and traces of leucine and tyrosine. Malic acid was previously only known as a vegetable product.

Of the watery secretion of the skin of amphibians little is known. The reaction of the secretion of the "mucous glands" is alkaline, while that of the "granular glands," [6] chiefly found on the dorsal surface of the flanks and legs, is acid. According to Leydig,[7] acrid substances are secreted in addition to mucin, in the case of the tree frog. In the case of the salamander and toad, poisonous substances have been separated.[8]

Gratiolet and Cloez [9] state that the poisonous substance in the skin glands of the toad and salamander is soluble in alcohol and of the nature of an alkaloid. Vulpian [10] and more recently Phisalix and Bertrand [11] have investigated this substance in the case of the toad. The symptoms of poisoning are—paralysis

[1] Bizio, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. xxxix. S. 33 ; Hofmann, *Wien. med. Wchnschr.*, 1873, S. 292 ; Bergmann, *St. Petersb. med. Ztschr.*, 1868, Bd. xiv. S. 28.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvii. p. 123.

[3] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 497.

[4] Weber, "Stud. ü. Saugethiere," Jena, 1886, S. 9.

[5] *Compt. rend. Acad. d. sc.*, Paris, 1886, tome ciii. p. 66 ; 1887, tome civ. p. 1292 ; and 1888, tome cvi. p. 1426.

[6] Hermann, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 291.

[7] *Arch. f. mikr. Anat.*, Bonn, 1875, Bd. xii. S. 119 ; and *Biol. Centralbl.*, Erlangen, 1892, Bd. xii. S. 458.

[8] Zalesky, *Hoppe-Seyler's Med.-chem. Untersuch.*, Berlin, 1866, Bd. i. S. 85 ; Casali, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1873, S. 64 ; Fornara, *ibid.*, 1877, S. 74.

[9] *Compt. rend. Acad. d. sc.*, Paris, 1852, tome xxxiv. p. 729.

[10] *Compt. rend. Soc. de biol.*, Paris, 1854, p. 135.

[11] *Arch. de physiol. norm. et path.*, Paris, 1893, Sér. 5, tome v. p. 511.

commencing in the hind-limbs, slowing and final arrest of the heart, and constriction of the pupil. Frogs, guinea-pigs, and small birds are killed by injection of the alcoholic extract of the collection of glands forming the so-called "parotids" of the toad. The poisonous substance is dialysable, and is probably an alkaloid. The blood of toads also contains small amounts of this substance, and the serum of a toad will kill a frog if introduced into the dorsal lymph sac.

The slime of fish is secreted by goblet cells in the epidermis, but has been little investigated from the chemical standpoint, on account of the great difficulty in obtaining it in sufficient quantities, and free from foreign substances. The slime of *Myxine glutinosa* is most easily obtained, and is found to contain a mucin-like body, which, however, does not yield a reducing sugar on boiling with dilute acid.[1]

The reaction of the skin of the eel and of *Myxine* is alkaline to litmus, but curiously does not affect phenophthalëin. A reducing sugar can be obtained from the slime of the eel by boiling with dilute acid.[2] According to Alcock,[3] the slime of the *Ammocœte* larva yields a proteolytic ferment on extraction.

(*b*) **Sebaceous secretions.**—Such secretions are formed by proliferation and subsequent degeneration of the cells lining the sebaceous glands, in which karyokinetic figures are frequent.[4]

These glands are present over the whole surface of the body, with the exception of the palms of the hands, soles of the feet, dorsal surface of the third phalanges, and glans penis.

Since it is impossible to collect sufficient quantities of the sebum of man for analysis, we have to rest content with analyses of the contents of sebaceous cysts, the *vernix caseosa* of the fœtus, or the contents of dermoid cysts of the ovary.

Glycerin and cholesterin fats, fatty acids, albumin (casein ?), free cholesterin and isocholesterin, with water and salts, are the main constituents of sebum.

The following table is taken from Hoppe-Seyler:[5]—

	Contents of a Distended Sebaceous Gland in Man.	*Vernix Caseosa* of Man.	Smegma Preputii of Man.	Smegma Preputii of Horse.
Water	317·0	669·8	...	...
Epithelium and albumin .	617·5	40·0	56·0	...
Fat	41·6	475·0	528·0	499·0
Fatty acids	12·1	...	...	...
Alcoholic extract . . .	...	150·0	74·0	96·9
Water extract . . .	...	33·0	61·0	54·0
Ash	11·8	...	...	...

[1] *Journ. Physiol.*, Cambridge and London, 1893, vol. xv. p. 488.
[2] Reid, *Phil. Trans.*, London, 1894, vol. clxxxv. p. 319.
[3] *Proc. Phil. Soc.*, Cambridge, 1891, vol. vii. pt. 5, p. 252.
[4] Bizzozero and Vasale, *Med. Chir. Centralbl.*, Wien, 1884, S. 77 and 179.
[5] "Physiol. Chem.," Berlin, 1881, Th. 4, S. 761.

Sotnitschewsky,[1] in an analysis of a dermoid cyst of the ovary, found tripalmitin, tristearin, and triolein; soaps of the acids of these fats and of caproic and caprylic acids, albumin, cholesterin, and an alcohol of high molecular weight, which, however, was not cetyl alcohol.[2] Tyrosine, hypoxanthin, xanthin, sugar, and glycogen were absent.

The *vernix caseosa* of man, according to Ruppel[3] and Liebreich,[4] contains cholesterin fats of oleic and palmitic acids, as well as glycerin fats, and also free cholesterin and isocholesterin.

The *cerumen* of the ear has been investigated by Petrequin,[5] and is found to contain potash soaps of oleic and stearic acids in the case of man and the ox, while in the dog the base is lime, and in the horse magnesia.

Wool fat, the sebaceous secretion of the sheep's skin, was proved by Hartmann[6] to contain no glycerin fats, but only those with cholesterin as alcohol. Schulze and Urich[7] confirmed this, and also found free cholesterin and isocholesterin.

These cholesterin fats (so-called "lanoline") have been specially investigated by Liebreich,[8] who finds that they are associated with keratinised structures, and are not necessarily formed in sebaceous glands, but may be formed within epidermic cells. Tortoiseshell, whalebone, horn, quills of porcupine and hedgehog, hoof of horse, and beak of crow, all contain these fats. The skin of the two-toed sloth has no sebaceous glands, and yet contains cholesterin fats, while pigeons bereft of their uropygial glands still have these substances in their feathers.

Such fats are peculiar, in that they can take up more than their weight of water, and also in that they do not become rancid, and offer a complete protection against the entrance of micro-organisms. Liebreich compares them to the wax of plants, which is an ether of a monohydric alcohol with a fatty acid.[9]

The secretion of the tail gland of the bird[10] has been chemically investigated by de Jonge.[11]

The secretion contains cetyl alcohol, the alcohol of spermaceti. No sugar or urea is present. Geese deprived of the tail gland and immersed in water are found to take up from two to two-and-a-half times as much water in their plumage as normal birds.[12]

The so-called "pigeons' milk," with which the young birds are fed by both parents during the earlier days of life, is practically a sebaceous secretion of temporary glands formed in the lateral pouches of the crop in both cock and

[1] *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 345.

[2] Ernst Ludwig (*Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. xxiii. S. 38) has quite recently found cetyl alcohol in the contents of dermoid cysts of the ovary.

[3] *Ibid.*, 1895, Bd. xxi. S. 122.

[4] "Verhandl. d. Berl. physiol. Gesellsch.," in *Arch. f. Physiol.*, Leipzig, 1890, S. 363.

[5] *Compt. rend. Acad. d. sc.*, Paris, 1869, tome lxix. p. 987; also 1869, tome lxviii. p. 940; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1871, Bd. i. S. 36; 1874, Bd. ii. S. 33.

[6] Inaug. Diss., Göttingen, 1868.

[7] *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1872, Bd. v. S. 1075; 1874, Bd. vii. S. 570.

[8] *Berl. klin. Wchnschr.*, 1885, Bd. xlvii. S. 761; *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvi. p. 1176; and "Verhandl. d. Berl. physiol. Gesellsch.," in *Arch. f. Physiol.*, Leipzig, 1890, S. 363.

[9] The secretion of the Harderian gland of the orbit of rodents, though fatty in nature, is not formed by disintegration of cells; Wendt, "Ueber die Harder'sche Drüse," Strassburg, 1877; Kamocki, *Biol. Centralbl.*, Erlangen, Bd. ii. S. 709.

[10] For anatomy, see Robby Kossmann, *Ztschr. f. wissensch. Zool.*, Leipzig, 1871, Bd. xxi. S. 568.

[11] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 225.

[12] Max Joseph, *Arch. f. Physiol.* Leipzig, 1891, S. 81.

hen. It contains fat, a proteid clotting with rennet, globulin, salts, and water, but is free from sugar.[1]

The secretion of the leg glands of lizards is probably of sebaceous nature.[2]

In certain fish, in addition to the secretion of slime by the goblet cells of the epidermis, a formation of fibrils takes place from specialised cells, termed "club cells," which may be distributed in the general epidermis or located in special glandular involutions, as in the Myxinoid fishes. This secretion reaches its highest development in the Myxinoids, and accounts for the extraordinarily tenacious slime of this class. The process has, however, also been observed in the case of the eel and lamprey.[3]

Mechanism of the Secretion of Sweat.

Goltz[4] in 1875 discovered the fundamental fact that excitation of the peripheral end of the divided sciatic causes the appearance of beads of sweat on the hairless pads of the hind-foot of the cat. He also saw the same effect in a dog. In the next year, Ostroumow,[5] and Kendall and Luchsinger[6] confirmed the result, and extended the details of the experiment.

Ostroumow showed that excitation of the abdominal sympathetic cord produced the same effect; that even after ligature of the aorta, sweat was still secreted upon excitation of the appropriate nerves; and, finally, that injection of atropine completely annulled the effect of such excitation.

Kendall and Luchsinger obtained the effect upon the fore-leg of the cat and dog, by exciting the nerves of the brachial plexus, confirmed the fact of the persistence of secretion after occlusion of the aorta, or crural artery in the case of the hind-limb, and further showed that, even after amputation of the leg, sweat could be produced on the pads of the foot for some fifteen to twenty minutes, by stimulation of the sciatic.

The fact that the production of sweat is an act of true secretion, and the existence of sudorific fibres having been demonstrated, the course of the fibres from the spinal cord next engaged attention.

The existence of sweat-fibres for the lower limb in the abdominal sympathetic cord, demonstrated by Ostroumow, was confirmed by Luchsinger[7] and Nawrocki,[8] and extended by both the latter observers[9] to the thoracic sympathetic cord, for the fore-limb. Later, sudorific fibres for the face, running in the cervical sympathetic, were demon-

[1] John Hunter, "Observations on Certain Parts of the Animal Œconomy," London, 1786, p. 191; Hasse, *Ztschr. f. rat. Med.*, 1865, Reihe 3, Bd. xxiii.; Claude Bernard, "Les liquides de l'organisme," Paris, 1859, tome ii. p. 232; Teichmann, *Arch. f. mikr. Anat.*, Bonn, 1889, Bd. xxxiv. S. 225; Charbonnel-Salle et Phisalix, *Compt. rend. Acad. d. sc.*, Paris, 1886, tome ciii. p. 286; Phisalix, *Compt. rend. Soc. de biol.*, Paris, 1890, Sér. 9, tome ii. p. 368; Reid, *Rep. Brit. Ass. Adv. Sc.*, London, 1894, p. 812.

[2] Leydig, "Die in Deutschland lebenden Arten der Saurier," 1872; Batelli, *Arch. f. mikr. Anat.*, Bonn, 1879, Bd. xvii. S. 346.

[3] J. Müller, "Untersuch. ü. die Eingeweide der Fische," Berlin, 1845, S. 11; Kölliker, *Würzb. med. Ztschr.*, 1860, Bd. i. S. 1; F. E. Schulze, *Arch. f. mikr. Anat.*, Bonn, 1867, Bd. iii. S. 137; Fœttinger, *Bull. Acad. roy. d. sc. de Belg.*, Bruxelles, 1876, Sér. 2, tome xli. p. 599; Blomfield, *Quart. Journ. Micr. Sc.*, London, 1882, vol. xxii. p. 355; Reid, *Phil. Trans.*, London, 1894, vol. clxxxv. p. 319.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. xi. S. 71.

[5] Ref. in *Jahresb. ü. d. Fortschr. d. Anat. u. Physiol.*, Leipzig, 1877, Bd. v.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xiii. S. 212.

[7] *Ibid.*, Bonn, 1877, Bd. xiv. S. 369.

[8] *Centralbl. f. d. med. Wissensch.*, Wien, 1878, S. 2.

[9] Nawrocki, *Centralbl. f. d. med. Wissensch.*, Berlin, 1878, S. 17; Luchsinger, *ibid.*, 1878, S. 36.

strated by Luchsinger[1] and Nawrocki,[2] in the horse and pig by the former observer, and in the pig by the latter. Both agreed that the fibres reach the sweat-glands of the face by the infra-orbital branch of the fifth cranial nerve, the junction being effected by branches from the cavernous plexus of the sympathetic. Neither of these investigators could satisfy himself of the presence of sweat-fibres in the facial nerve.

The origin of the sudorific fibres in the spinal cord has been studied by Luchsinger, Nawrocki, Vulpian, Ott,[3] and more recently by Langley.[4]

In the case of the hind-limb of the cat, according to Langley, the sudorific fibres enter the sympathetic cord by the white *rami communicantes* of the last two thoracic and first three or four lumbar nerves, become connected with nerve-cells in the sixth and seventh lumbar, and first and second sacral ganglia of the sympathetic, and leave by the grey rami of these ganglia, to enter the anterior divisions of the corresponding spinal nerves, and so the sciatic. The first and second lumbar spinal nerves seem to supply the greatest number of secretory fibres. The grey ramus to the sixth lumbar nerve is found to chiefly supply the sweat-glands of the inner part of the foot, that to the second sacral nerve the outer part, and, in the main, the successive rami from above downward supply strips of the skin of the foot from within outwards, though considerable, and, in different individuals, varied overlapping of fields is noted.

In the case of the fore-limb, the same observer finds that the sweat-nerves are supplied to the sympathetic chain by the fourth to the ninth thoracic spinal nerves, the main outflow of fibres being usually found in a nerve near the middle of the series. All these fibres run up in the sympathetic cord to the *ganglion stellatum*, where a connection with nerve-cells is effected, and by the grey rami of this ganglion reach the brachial plexus,[5] and so the median and ulnar nerves for their final distribution.

The grey rami to the sixth and seventh cervical nerves seem to chiefly supply the inner part of the fore-foot, while that to the first thoracic nerve chiefly supplies the outer part.

The fibres for the face, according to Nawrocki, leave the cord by the second, third, and fourth anterior roots, and run up in the cervical sympathetic to finally reach the infra-orbital branch of the fifth cranial nerve, *viâ* the cavernous plexus.

Vulpian[6] and Ott[7] maintained that, in addition to the sudorific fibres supplied to the limbs *viâ* the sympathetic, others are supplied directly from the cord with the nerves forming the limb plexuses. The existence of such fibres was denied by Nawrocki, and Langley fully confirms the statements of this observer.

Furthermore, Vulpian and Ott maintained that inhibitory fibres to the sweat-glands exist, and the theory has been recently revived by Arloing.[8]

Vulpian's evidence for the existence of such fibres was, that contem-

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxii. S. 126.

[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1880, S. 945.

[3] *Compt. rend. Acad. d. sc.*, Paris, 1878, tome lxxxvi. pp. 1308 and 1434; Ott, *Journ. Physiol.*, Cambridge and London, 1879, vol. ii. p. 42.

[4] *Ibid.*, 1891, vol. xii. p. 347; *ibid.*, 1894, vol. xvii. p. 296.

[5] Eckhard (*Arch. f. Anat., Physiol. u. wissensch. Med.*, Berlin, 1849, S. 427) quotes a case in man where contusion of the brachial plexus led to continuous sweating of the hand on the side of the injury.

[6] *Loc. cit.* [7] *Loc. cit.*

[8] *Arch. de physiol. norm. et path.*, Paris, 1890, Sér. 5, tome ii. p. 1; and 1891, Sér. 5, tome iii. p. 241.

poraneous excitation of cut sciatic and abdominal sympathetic causes less sweat on the pads of a cat's feet than excitation of sciatic alone, and the sweat-stimulating drug pilocarpine causes more sweating when sciatic or sympathetic are cut than intact.

Later, Vulpian[1] abandoned this theory. He was led to the idea of the existence of inhibitory fibres in the cervical sympathetic by consideration of the old experiment of Dupuy,[2] in which section of the cervical sympathetic in the horse leads to sweating on the face on the side of section. Mere excess of blood supply to sweat-glands, from the vaso-dilation which occurs simultaneously, is probably *per se* no stimulus to the action,[3] but there is no doubt that the excitability of the glands is thereby raised, and if, with Luchsinger,[4] it is admitted that a few sweat-fibres originate with the fifth cranial nerve, the result is simply due to painful reflex, for Luchsinger got no sweating on section of the sympathetic in the neck of a chloralised horse, though stimulation of the peripheral end gave abundance.

The evidence adduced by Ott is the immediate cessation of a secretion previously evoked by pilocarpine, on excitation of the peripheral end of the divided sciatic. Even if it were admissible that the accompanying vasomotor constriction could cause the effect (which it is not, seeing that in the amputated foot sweat can still be called forth), the result, he maintains, is obtained too suddenly to be accounted for in this manner.

Again, he states that *irritation* of the abdominal sympathetic causes a dryness of the pads of the foot on the side of irritation, and that pilocarpine accentuates the difference in condition between the foot on the side of irritation and the normal foot on the opposite side.

Finally, division of the abdominal sympathetic produces moist pads on the side of section, and injection of pilocarpine makes these pads sweat before the others.

In Arloing's experiments on oxen and donkeys, the cervical sympathetic is divided, and time is allowed to elapse until the vaso-dilation has passed off. Pilocarpine now produces more marked secretion on the side of section, which is interpreted as meaning that inhibitory impulses, restraining the action of the glands on the sound side, have been removed on the side of section.

It has always been a matter of difficulty to differentiate the action of two oppositely acting sets of fibres running in the same nerve-trunk, and it must be admitted that the evidence so far for the existence of inhibitory fibres for sweat secretion is not strong.

Excitation by appropriate stimuli of the regions of the spinal cord from which the sweat-fibres emerge leads to an outpouring of sweat on the parts of the skin supplied by these fibres. Thus, if the spinal cord is divided above the exit of the twelfth thoracic nerve in the cat, and the animal exposed to heat (60° to 70° C. for five to ten minutes), sweating still occurs on the hind-limbs.[5]

Nawrocki[6] and Marmé[7] denied this effect, and maintained that it is only when there is continuity of the cord with the bulb that such stimulation causes sweating. Later, however, Nawrocki[8] obtained the

[1] Vulpian et Raymond, *Compt. rend. Acad. d. sc.*, Paris, 1879, tome lxxxix. p. 11; *Rev. internat. d. sc. biol.*, Paris, 1880, p. 115; and "Leçons sur les substances tox. et médic.," tome i. pp. 148–149.

[2] *Journ. de méd., chir., pharm.*, etc., Paris, 1816, tome xxxvii.

[3] But see Levy, "Verhandl. d. Berl. physiol. Gesellsch.," in *Arch. f. Physiol.*, Leipzig, 1892, S. 155.

[4] *Tagebl. d. Versamml. deutsch. Naturf. in Baden-Baden*, 1879.

[5] Luchsinger, *loc. cit.*

[6] *Centralbl. f. d. med. Wissensch.*, Wien, 1878, S. 17.

[7] *Nachr. v. d. k. Gesellsch. d. Wissensch. u. d. Georg.-Aug. Univ.*, Göttingen, 1878, p. 102.

[8] *Centralbl. f. d. med. Wissensch.*, Berlin, 1878, S. 721.

result in a few cases with divided cord. Obviously a positive case in such an experiment is worth many negative, since the excitability of the cord below the section may possibly be depressed at the time of making the test. It is generally accepted that spinal "sweat-centres" exist.

On the other hand, no cerebral centres for sweating have yet been experimentally demonstrated.[1]

According to Levy Dorn,[2] the spinal "sweat-centres" are very resistant to the action of cold. In cats cooled till the rectal temperature was 22° to 28° C., sweating was still obtained by reflex excitation or dyspnœa, but heating (70° C.) caused little sweating, the cooled cat being as it were "protected," in that the heat which is to restore it, does not, when applied, immediately call forth a reflex outpouring of sweat, by the subsequent evaporation of which, heat would be abstracted from the body.

The nervous mechanism of sweat secretion may be called into action by central stimuli, by reflex action, or by peripheral stimuli. A venous condition of the blood is one of the most active stimuli to the central mechanism, and one frequently employed in experimental work. If an animal be partially asphyxiated, after section of the spinal cord in the mid-dorsal region, sweat breaks out on the pads of the hind-feet, even after division of all the posterior roots behind the section.[3]

Raising the temperature of the blood produces a similar effect, and the result is also obtained with divided posterior roots, and hence is not reflex; moreover, the effect is stopped by section of the sciatic, and hence is not of peripheral origin as a result of heating of the terminal apparatus.

Certain drugs, especially picrotoxin and strychnia, appear to cause sweating exclusively by their action on the spinal cord. Nicotine and eserine cause slight sweating after section of the limb nerves, and are therefore not exclusively, though mainly, central stimulants.[4]

Reflexly, it may be broadly stated that stimulation of almost any afferent channel will cause sweating. A cat will sweat on the pads of its feet at the sight of a dog, mustard in the mouth causes sweat on the foreheads of many persons, and the application of heat to the skin is a familiar cause of increased action of the glands. According to Greidenberg,[5] in a patient with sweating legs, slight skin stimuli diminished the secretion, while strong stimuli caused an increase.

Directly from the periphery, the sweat-glands may be excited by certain drugs or by raising their temperature.

Pilocarpine excites secretion of sweat after complete division of the nerves, and localised secretion may be produced by introducing it beneath the skin. Its action is probably in the main upon the terminations of the nerves in the glands, since it is, as a rule, non-effective, when sufficient time has been allowed to elapse after section of the nerves to ensure complete degeneration (Luchsinger, Nawrocki, and Vulpian). On the other hand, Max Levy[6] states that pilocarpine may still give good

[1] Bloch, "Thèse de Paris," 1880.

[2] "Verhandl. d. Berl. physiol. Gesellsch.," in *Arch. f. Physiol.*, Leipzig, 1895, S. 198.

[3] Luchsinger, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 369.; Robillard, "Thèse de Doct.," Lille, 1880.

[4] Luchsinger, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xv. S. 482; Högyes, ref. in *Jahresb. ü. d. Fortschr. d. Anat. u. Physiol.*, Leipzig, 1881, Bd. ix. S. 72.

[5] *Jahresb. ü. d. Fortschr. d. Anat. u. Physiol.*, Leipzig, 1882, Bd. x. S. 81.

[6] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892, Bd. v. S. 68.

secretion, when excitation of previously divided nerves is without effect, pointing to stimulation of the gland protoplasm by the drug.

According to Rossbach,[1] small doses act upon the nerve-endings, while large doses also affect the gland protoplasm; and some of the experiments of Luchsinger, Marmé, and Högyes, in which pilocarpine caused secretion, long after the time necessary for complete degeneration of the nerves had elapsed, point to the same conclusion.

There appears to be no central action by pilocarpine, for Robillard,[2] after separating the foot of a cat from the body, with the exception of the tibial nerve, obtained no secretion of sweat on injection of pilocarpine into the general circulation; though the nerve was proved to conduct, by a profuse sweat caused on asphyxiation.

Muscarine[3] also acts as a peripheral excitant, but is less active than pilocarpine.

Atropine and duboisine are both antagonistic to pilocarpine and muscarine.

In the cat an injection into a vein of 3 mgrms. of atropine is sufficient to make stimulation of the sciatic ineffective; subsequent intravenous injection of 10 mgrms. of pilocarpine will cause sweating, though the nerve is still without action on excitation. In such a case the atropine poisons the nerve-ending, but the gland protoplasm is still excitable and responds to pilocarpine. According to Rossbach,[4] a dose of 20 to 30 mgrms. of atropine is needed, in the case of a cat, to paralyse the gland-cells to such an extent that subsequent local application of pilocarpine is without effect. All glandular apparatus appears to be far more sensitive to atropine than to pilocarpine.

The local paralysing effect of atropine was elegantly demonstrated by Aubert.[5] If the palm or finger (carefully cleaned) is pressed on to paper sensitised with silver nitrate, the spots of chloride formed at the mouths of the sweat-ducts are quite visible. If the experiment is tried, after a pad soaked in atropine solution has been tied over a limited surface overnight, that surface is found to yield no spots, in contrast to the surrounding field.

Finally, the terminal sweat apparatus is very sensitive to change of temperature. Luchsinger[6] has shown that not only cold but excessive heating retards the action of the glands. Thus if, on a warm day, one hand be held in water at 45° to 50° C. for ten minutes, while the other is immersed in water at 15° to 30° C., and exercise is then taken, the hand which was in water at the lower temperature commences to sweat at once, the other not for some considerable time. In experimental work, in which the excitation of nerves is undertaken and the outbreak of sweat observed, the greatest caution is necessary to keep the temperature of the extremities constant, for with a cold foot a nerve root holding sweat-fibres in reality, may be wrongly considered to hold none, if the terminal apparatus is depressed by cold.

That the formation of sweat is a true act of secretion, and not merely filtration, is shown by experiments already quoted, in which it is noted that after stoppage of the circulation sweat is still secreted on

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxi. S. 1. [2] *Loc. cit.*

[3] Trümpy and Luchsinger, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii. S. 501; Ott and Wood Field, *Journ. Physiol.*, Cambridge and London, 1878, vol. i. p. 193; Högyes, *loc. cit.*

[4] *Loc. cit.* [5] *Lyon méd.*, 1874.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii. S. 478.

excitation of nerves; and further, by the fact that by means of atropine the secretion of sweat can be stopped in spite of the continued circulation of the blood.

It is probably right to conclude that the blood supply is a necessary adjuvant to the prolonged activity of the gland-cells, but not the stimulant to their action, though, according to Levy,[1] secretion is provoked upon reinstallation of the circulation, in a limb with cut sciatic, which has been long kept anæmic. This effect may possibly be due to the mechanical stimulation of the glands by the pulse.

Levy Dorn[2] placed the hind-limb of a cat in a receptacle within which the air pressure could be raised, and found that the secretion could overcome a pressure in excess of that in the large arteries.

Nothing is definitely known as to the existence or not of any action of the nervous system upon the sebaceous glands.

According to Arloing,[3] section of the cervical sympathetic in donkeys causes exudation of sebum from the sebaceous glands of the skin of the ear, reaching its maximum fifteen hours after section, and lasting for sixty-four hours. Stimulation of the peripheral end of the nerve also causes secretion from these glands.

The glands of the skin of the frog undergo periodic contraction and expansion by means of their muscular sheaths,[4] and have been carefully studied by Engelmann,[5] Stricker and Spina,[6] and Drasch,[7] in the web and *membrana nictitans*. The spontaneous movements in the case of the web glands are stopped temporarily by section of the sciatic, or seventh, eighth, and ninth anterior spinal roots. Excitation of the sciatic or reflex stimulation of the skin leads to contraction of the glands, as also does direct excitation by vapours of chloroform or ether, or by carbonic acid gas. During contraction of the whole gland, by its muscular sheath, the lining gland-cells swell, and, according to Drasch, in the case of the *membrana nictitans*, the fifth cranial nerve, on excitation, causes contraction of the sheath only, while excitation of the sympathetic causes swelling of the cells. Pilocarpine causes increased secretion by these glands. Stricker and Spina advanced a theory of secretion based upon observations of these glands, maintaining that, in the act of swelling, fluid is sucked in by the cells from the surrounding lymph spaces, and on contraction forced out into the lumen; the theory obviously involves the assumption of some valvular structure in the protoplasm, of which we know nothing, and furthermore has been disposed of by Drasch, who has found that the glands of the *membrana nictitans* may secrete freely in stages of immobility of the lining cells.

In the case of fish—in the eel it has been shown that the secretion of the goblet cells of the epidermis and of the club cells (when present) is under the influence of the nervous system, but the nerve paths have not been worked out.[8]

Electro-motive Phenomena in Skin Glands.

In attempting to demonstrate the existence of currents in the uninjured muscles of the frog, du Bois Reymond[9] discovered that the

[1] *Loc. cit.*
[2] "Verhandl. d. Berl. physiol. Gesellsch.," *Arch. f. Physiol.*, Leipzig, 1893, S. 383.
[3] *Arch. de physiol. norm. et path.*, Paris, 1891, Sér. 5, tome iii. p. 241.
[4] Ascherson, *Arch. f. Anat. u. Physiol.*, Leipzig, 1840, S. 15.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 498.
[6] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1880, Bd. lxxx. Abth. 3, S. 95.
[7] *Arch. f. Physiol.*, Leipzig, 1889, S. 96.
[8] Reid, *Phil. Trans.*, London, 1894, vol. clxxxv. p. 319.
[9] "Untersuch. ueber thierische Elektricität," Bd. ii. Abth. 2, S. 9–20.

skin itself is a seat of electro-motive force, which he located in the glands. The current is in the direction from the free to the deep surface, the former being electrically negative to the latter. A current so oriented may be termed "ingoing," and is the normal direction of the "current of rest" of all secreting membranes, so far investigated.

The discovery was corroborated by Rosenthal,[1] and extended to the case of the stomach and gut mucosæ in the frog and rabbit, while Hermann[2] found a similar current in the skins of many fish, and, more recently, in the tree frog, proteus, and axolotl.[3] Such currents as a rule exhibit spontaneous variations in intensity, especially in the case of the skin of the frog.

Valentin,[4] and later Roeber,[5] furthermore found that in the case of the frog's skin excitation of the cutaneous nerves causes a variation in the electro-motive force of the resting skin, and the latter observer that this phenomenon could be produced by reflex excitation, and was not abolished by curare.

The direction of the "current of action" evoked by excitation of nerves was not found to be constant by Roeber, a fact corroborated by all subsequent investigators. Thus Engelmann[6] observed a double excitatory variation of the "current of rest," namely, an outgoing followed by an ingoing current (negative followed by positive variation), while Hermann[7] noted an ingoing "current of action," often preceded by an outgoing current of short duration, and Bayliss and Bradford[8] state that it is "scarcely possible to speak of a normal excitatory variation."

Hermann and Luchsinger[9] found that the cat's foot also gave an ingoing "current of rest," but that the current developed on excitation of the sciatic was constantly ingoing, and prevented from development by the exhibition of atropine.

Luchsinger[10] demonstrated the existence of exactly similar currents in the snout of the pig, goat, cat, and dog on excitation of the cervical sympathetic or infra-orbital nerve, and Hermann and Luchsinger[11] in the tongue glands of the frog, though in the latter case excitation of the hypoglossal or glossopharyngeal nerve gave a triphasic "current of action," an outgoing being interpolated in a long lasting ingoing phase. Tarchanoff[12] has further indicated that parts of the skin of man rich in sweat-glands (*e.g.* palm of hand), are negatively electrical to parts poor in sweat-glands (*e.g.* skin over deltoid), and that the ingoing "current of action" of such glands can be excited reflexly by very slight stimuli, such as sound or even the expectation thereof, odours, or mental effort.

The well-known *Willkürversuch* of du Bois Reymond, in which, when the index-fingers of the two hands are immersed in vessels of liquid in circuit with a galvanometer, a voluntary effort of the

[1] *Arch. f. Physiol.*, Leipzig, 1865, S. 301.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxvii. S. 280.
[3] *Ibid.*, 1894, Bd. lviii. S. 242.
[4] *Ztschr. f. rat. Med.*, 1861, Bd. xv. S. 208.
[5] *Arch. f. Physiol.*, Leipzig, 1869, S. 633.
[6] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 97.
[7] *Ibid.*, 1878, Bd. xvii. S. 291 ; and 1882, Bd. xxii. S. 280.
[8] *Journ. Physiol.*, Cambridge and London, 1886, vol. vii. p. 223.
[9] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 310.
[10] *Ibid.*, 1880, Bd. xxii. S. 152.
[11] *Ibid.*, 1878, Bd. xviii. S. 460.
[12] *Ibid.*, 1890, Bd. xlvi. S. 46.

muscles of one forearm (*e.g.* grasping a rod) gives a current passing up the contracting arm, is probably to be explained by the concomitant excitation of the sweat-glands on the side of action.

Not only in the case of membranes containing complex glands is an ingoing "current of rest" observed, but also in secreting membranes supplied with unicellular glands (goblet cells), as the pharynx and cloaca of the frog,[1] or the skin of the fish;[2] and furthermore, in certain membranes, quite free of secretory structures, and covered only by stratified epithelium, such as the skin of the pigeon and the mucosa of the crop of the same bird in winter.[3]

The attempts to explain the causation of the above currents, it must be confessed, have not been very satisfactory.

As regards the constantly observed ingoing "current of rest," it is obviously of the first importance to determine whether it is of purely epidermic origin, of purely glandular origin, or whether it receives a component from both sources. The experiments with the non-glandular skin of the bird above-mentioned show that simple stratified epithelium can give rise to such a current, and Hermann[4] has further proved that shaving the epidermis of the cat's foot lowers the electro-motive force of the "current of rest." Furthermore, Bach and Oehler[5] found that pencilling the skin of the frog with solution of corrosive sublimate abolished the "current of rest," though the excitatory change from the glands beneath could still be obtained by exciting the nerves of the skin.

On the other hand, it can hardly be denied that in such cases as the gastric mucosa of the frog, where the epithelium is practically all converted into unicellular glands (goblet cells), the marked ingoing "current of rest" is of glandular origin,[6] and this must also be the case in such a membrane as the cloacal mucosa of the frog.

It is simplest, in the present state of knowledge, to admit that both stratified epithelium and gland protoplasm can give rise to currents.

Hermann and Biedermann consider such currents due to alteration of metabolic activity in the continuity of protoplasm. Protoplasm becoming "altered" to mucus in a goblet cell is negative electrically to the unaltered material at the base of the cell, and the same in the process of keratinisation in the continuity of epithelium. Since altered parts are negative electrically to unaltered or less altered, the result will be an ingoing current, whether we choose the glands or the epidermis, or both, as the source of the electro-motive force of the "current of rest."

If we turn to the case of the "action current," it is only in the case of the mammalian glands that any clear explanation on the above hypothesis is feasible. In these glands, as already mentioned, the "current of action" is purely ingoing, and it is only necessary to assume that in action the "difference" between the base and the free border of the cells becomes more marked than at rest, with a concomitant development of electro-motive force with ingoing current.

In the skins and other secreting membranes of amphibians and fish, we are met with the difficulty that it is not possible to predict with certainty what will be the direction of the "action current" elicited by

[1] Biedermann, *ibid.*, 1893, Bd. liv. S. 209.
[2] Hermann, *loc. cit.*; Reid, *Phil. Trans.*, London, 1893, Bd. clxxxiv. p. 335.
[3] Reid, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 359.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 242.
[5] *Ibid.*, 1880, Bd. xxii. S. 30. [6] Bohlen, *ibid.*, 1894, Bd. lvii. S. 97.

excitation of the nerves. The strength of the stimulus, and the extent to which the normal ingoing "current of rest" is developed, affects the result, and hypotheses have been based upon both of these factors of the case.

Hermann[1] long ago suggested the possibility of augmenting and inhibitory fibres to the glands, and in his most recent publication[2] still entertains the idea. On the other hand, Biedermann suggests that the two sides of protoplasmic activity (the katabolic and anabolic) in the secretory cells are associated with generation of electro-motive force, causing currents in opposite directions in the two cases; that the electro-motive force of the "current of rest" is the algebraic sum of these opposing forces at the moment; and that the results of nerve excitation are related directly to the ascendancy of one or the other metabolic action at the time of stimulation.

The production of an outgoing "current of action" is considered by Biedermann as due to the nerve excitation provoking an excess of anabolic action in the cell, that of an ingoing "current of action" as due to excess of katabolism, so that one and the same class of nerve-fibre is supposed to produce quite opposite results in the cell, the effect being partly conditioned by the state of the balance in the cell between the two processes at the moment of excitation, and partly by the strength of the stimulus. He supposes that the cell process least developed at the time of excitation, tends to be stimulated in excess of its fellow, so that if the ingoing "current of rest" is weak, as a result of slight katabolic ascendancy, excitation tends to cause an ingoing "current of action"; and, *vice versâ*, if the ingoing "current of rest" is strong, as a result of marked katabolic ascendancy, the result of excitation of the cell is liable to be the development of an outgoing "current of action."

Hermann objects to this, that if the electrical sign of excess of anabolism over katabolism is plus, the induction of such a condition must start from the deep ends of the cells, *i.e.* from the ends from which they get their pabulum from the blood, and excess of positivity of this end of the cell comes, so far as the direction of current is concerned, to the same thing as excess of negativity at the free end of the cell, associated by hypothesis with katabolic ascendancy, and should develop a current in the same direction, *i.e.* ingoing.

It may also be noted in this connection, that, according to Bohlen,[3] in the gastric mucosa of mammals, cessation of circulation or any interference with blood supply tends to convert the normal ingoing into an outgoing "current of rest." If an outgoing current is associated with excess of anabolism over katabolism, it is difficult to conceive how withdrawal of blood supply can induce such a change. A similar complete reversal of the direction of the "current of rest" is obtainable in the secreting membranes of the frog and fish by abstraction of heat, or by narcotisation with carbonic acid gas, ether, or chloroform.

A strong stimulus of a nerve trunk may, in practice, cause an outgoing action current, and a weak stimulus one that is ingoing, but it is again difficult to conceive that difference in the strength of stimulus of one class of nerve-fibre can alter the whole character of the metabolism in the cells.

Hermann was at one time of opinion that the two kinds of glands in the frog's skin might be associated with the two phases of the excitatory variation; and the lip of the eel, which contains no club cells but only goblet cells, gives an outgoing "action current," while the body skin, rich in club cells and poor in goblets, gives an ingoing "action current" with the same strength of stimulus;[4] but since the cloacal or pharyngeal mucosa of the frog, containing only one sort of secretory cell, and the non-glandular crop of the winter pigeon, can give currents in both directions, the hypothesis is not of universal application.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvii. S. 303.
[2] *Ibid.*, 1894, Bd. lviii. S. 242.
[3] *Loc. cit.*
[4] Reid and Tolputt, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 203.

Absorption by the Skin.

Man.—To decide the case for or against the possibility of absorption by the human skin, would appear a simple problem, yet a literature reaching back over a century indicates that the production of unimpeachable testimony on either side has proved a matter of no little difficulty.

A fluid in contact with the skin is separated from the blood vessels by layers of epidermic cells with intercellular spaces, but since the superficial cells (except in the palm of the hand and sole of the foot) are greasy with sebum, one of the first conditions for absorption is that the fluid shall be able to wet the surface, so that imbibition by the cells, or entrance of the fluid into the capillary spaces between them, may take place. Though lanoline, the natural fat of the skin, takes up water, such action only occurs slowly, and unless the skin is soaked long in warm water, it is a familiar observation that it does not easily become sodden, except in the case of the palms and soles. It is therefore not to be expected that water or watery solutions will be capable of absorption by the skin of man, and the experimental evidence is distinctly against such an assumption.

The method of some of the older observers, of attempting to decide the question of absorption of water by immersing a man in a bath after weighing, and weighing again after a prolonged sojourn therein, we may dismiss by a bald statement of obvious sources of error.

(*a*) There is no guarantee that the normal loss of weight of the body per unit time, through lungs and skin, is the same during the bath as estimated during preceding hours. (The experiments showed, in different instances, gains, losses, and absence of change of weight.)[1]

Further, mere soakage of the epidermis of palms and soles may mask an actual loss of weight in the bath.[2]

(*b*) It is impossible to be certain that the epidermis of the whole body is devoid of fissures through which water might reach the deeper parts.

(*c*) It is difficult to totally exclude absorption by immersed mucous surfaces.

(*d*) A balance sensitive enough to indicate a difference of a few grammes on a weight of many kilos., is difficult to construct.

(*e*) A considerable loss of surface epidermis occurs in "drying" the body with a towel.

An improvement upon the method of total immersion is that of immersion of a part of the body, but the vessel, instead of being weighed before and after immersion of the part of the body, as in the experiments of Vierordt and Eichberg,[3] is better graduated as in the experiments of Falck,[4] or provided with a capillary pipette, by means of which absorption can be determined by fall of level of fluid,[5] because, by the gravimetric method, the error from mere soakage of epidermis becomes far larger than in the volumetric method, though here also a slight diminution in volume accompanies imbibition by the palm or sole,

[1] Jamin et de Laurés, *Compt. rend. Acad. d. sc.*, Paris, 1872, tome lxxv. p. 60.
[2] Poulet, *ibid.*, 1856, tome xlii. p. 435.
[3] *Arch. f. physiol. Heilk.*, Stuttgart, 1856. [4] *Ibid.*, 1852.
[5] Madden, "An Experimental Inquiry into the Physiology of Cutaneous Absorption," Edinburgh, 1838; Fleischer, Inaug. Diss., Erlangen, 1877.

if an arm or leg be used, since the combination of a body with water in which it is soaked is accompanied by contraction, so that the total volume after soakage is less than the sum of the initial volumes.[1]

Fleischer could obtain no positive evidence of absorption of water by the skin of the arm, immersed in a Mosso's plethysmograph (provided with a capillary pipette) for three hours.

Solutions of chemical substances easily detected in the secretions have been much employed, a part of the body being immersed, or the solution applied by means of a spray. Colouring matters, inorganic salts, and drugs with marked physiological action, have been used. In such experiments the chief points to be observed are—(*a*) Integrity of the epidermis before the experiment, and absence of destructive chemical action by the substance used during its course; (*b*) absolute exclusion of possibility of absorption by the lungs in the case of a volatile substance, or of a salt yielding a volatile substance under the action of the sweat; (*c*) the choice of substances capable of recognition with certainty in minute quantities in the secretions.

Braune,[2] using foot baths of solutions of potassium iodide, iodine, and hydriodic acid, with a layer of oil over the surface of the solution, was unable to detect iodine in the secretions. Parisot,[3] using baths of watery solutions of potassium iodide and ferrocyanide, belladonna, digitalis, and the colouring matter of rhubarb, repeated twice a day for three to eight days, obtained no evidence of absorption. Hüfner[4] found no lithium by the spectroscope in the urine after foot baths of lithium chloride. V. Wittich[5] and Fleischer[6] were unable to confirm Rohrig's[7] statement, that aqueous solutions of potassium iodide are absorbed. Winternitz[8] could get no evidence of absorption of 10 to 15 per cent. solutions of lithium chloride in water, and results with cocaine were negative.[9]

Again, Fubini and Pierini[10] could get no evidence of absorption of the following solutions:—Potassium ferrocyanide, 3 per cent.; santonate of soda, 2 per cent.; salicylate of soda, 5 per cent.; potassium iodide, 5 per cent.; and lithium benzoate, 2 per cent., all dissolved in water.

Hence it is probably correct to conclude that watery solutions not acting chemically upon the epidermis, and water itself, are not capable of absorption by the intact skin of man.

If we now turn to the case of fluids that can wet the skin, such as chloroform, ether, alcohol, etc., we find that a certain amount of evidence of absorption is obtainable in the case of man.

Since chloroform, though an excellent fat solvent, causes pain and blistering when long in contact with the skin of man, the experiments have been mostly made with ether and alcohol. Ether is a better solvent of fats than alcohol, and hence is more likely to give positive results. Krause[11] maintained that both alcoholic and ethereal solutions of salts are absorbed by the skin, but Fleischer,[12] using a volumetric

[1] Quincke, *Arch. f. d. ges. Physiol.*, Bonn, 1870, Bd. iii. S. 332.
[2] Diss., Leipzig, 1856.
[3] *Compt. rend. Soc. de biol.*, Paris, 1863, tome lvii. p. 327.
[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1880, Bd. iv. S. 378.
[5] Hermann's "Handbuch," Leipzig, 1881, Bd. v. Th. 2, S. 257.
[6] *Loc. cit.* [7] "Die Physiologie der Haut," Berlin, 1876.
[8] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 405.
[9] See also Soulier, "Traité de therapeutique et de pharmacologie," 1891, tome i. p. 385.
[10] *Arch. ital. de biol.*, Turin, 1893, vol. xix. p. 357.
[11] Wagner's "Handwörterbuch," 1844, Bd. ii. S. 174. [12] *Loc. cit.*

method, could observe no absorption of absolute alcohol by his own skin in an hour and a half, and Ritter[1] denies entirely the absorption of alcohol or alcoholic solutions of salts by the human skin. Winternitz[2] got spectroscopic evidence of lithium in the urine after keeping the skin of the arm in contact with an ethereal solution (with a little added alcohol) for three and a half hours, but missed the effect with a purely alcoholic solution. The last-mentioned observer also denies the statement of Parisot,[3] that solutions of atropine in alcohol and chloroform, applied to the forehead, cause mydriasis.

It would appear that previous removal of the grease of the skin by ether allows a slight absorption of watery solutions to take place, for Winternitz[4] got traces of lithium in the urine on applying a watery solution of the chloride to the skin cleaned with ether, but not till nine hours after the application.

If a substance applied to the skin is volatile at the temperature of the body, the vapour may possibly pass into the capillary spaces between the epidermic cells, and dissolve in the fluid in the sweat ducts, and so finally reach the blood vessels, and be absorbed; but in experiments with such substances the greatest precautions must be taken to exclude absorption by the respiratory tract, and again with human skin the results of different observers are conflicting. Rohrig's[5] positive results with tincture of iodine are denied by Fleischer,[6] who, wearing a mask with a tube to the outer air, found no iodine in the urine up till six hours after an application to the skin of the back for one and a half hours. Next morning Fleischer found iodine in the urine, but this may have been absorbed by the lungs during sleep, or the result of the destructive action of the substance on the epidermis. Mesnil,[7] placing the arm in a Mosso's plethysmograph, filled with vapour of iodine, could get no evidence of absorption after thirty-two hours' exposure. On the other hand, guaiacol is asserted by several observers to be absorbed.[8]

Oily solutions and unguents, since they "wet" the skin, one would expect to be capable of absorption, but such substances are viscous and must be mechanically forced into the intercellular spaces and hair follicles, if any marked effect is to be obtained. According to Winternitz,[9] the mere application of oily solutions of veratrine and aconitine to the skin of man is without effect. Baschkis and Obermayer[10] obtained evidence of presence of lithium in the urine three hours after rubbing in an ointment of lithium carbonate, oleic acid, and lanoline, but Fleischer[11] could not obtain evidence of absorption of unguents holding potassium iodide, veratrine, morphia, quinine, and salicylate of soda, nor could Fubini and Pierini[12] find salicylic acid in urine after painting a solution in oil of almonds on the hand and forearm.

But the most important case is that of mercurial ointment, which is undoubtedly absorbed into the system. In this, in addition to fine

[1] Diss., Erlangen, 1883.
[2] *Loc. cit.*
[3] *Compt. rend. Acad. d. sc.*, Paris, 1863, tome lvii. p. 327.
[4] *Loc. cit.*
[5] *Loc. cit.*
[6] *Loc. cit.*
[7] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1894, Bd. vii. S. 775, ref.
[8] Sciolla, "Cronaca della clinica medica di Genova," 1892–93, p. 191; Linossier and Lannois, *Compt. rend. Soc. de. biol.*, Paris, 1894, pp. 108–110 and pp. 214–215; Guinard and Stourbe, *ibid.*, 1894, pp. 180–182.
[9] *Loc. cit.*
[10] *Centralbl. f. klin. Med.*, Bonn, Bd. xii. S. 65.
[11] *Loc. cit.*
[12] *Loc. cit.*

globules of mercury, there is present the black oxide of the metal,[1] and it is probable that, after formation of calomel by the sodic chloride of the sweat, in the presence of oxygen a further conversion into corrosive sublimate takes place, which is finally taken up by the blood.

Though evidence of vaporisation of mercury in mercurial ointment, at the body temperature, can be got by hanging a gold leaf over the preparation, such vapour cannot of course pass through wet capillary walls into the blood. The theory also of a passage of the fine globules of mercury through into the blood is denied by Bärensprung,[2] Hoffmann,[3] and Rindfleisch,[4] though the fine particles are certainly mechanically forced into the hair follicles, sweat ducts,[5] and the interstices of the superficial epidermic cells, thence to gradually undergo removal.

Finally, mention may be made of the fact that by taking advantage of the cataphoric action of the galvanic current (so-called electro-osmose),[6] it is possible to force watery solutions into the capillary spaces between the epidermic cells, and so artificially cause absorption, either by subsequent diffusion into the blood vessels, or by the recoil of distended spaces forcing fluid into lymphatic channels.[7] The direction in which the fluid is moved is that of the electrical current, and the quantity carried through a porous partition is directly proportional to the intensity of the current, but organic membranes are far less permeable than porous earthenware.[8]

It is not then to be expected that the effects with human skin will be very marked, since, in practice, only a few milliampères can be passed with comfort to the patient.

Munk[9] got evidence of iodine and quinine in the urine, with positive electrodes of modeller's clay moistened with potassium iodide, and quinine in aqueous solution. Herzog[10] anæsthetised the skin with cocaine solution on the positive electrode, when mere application without passage of current was without effect, as also was passage of current without cocaine.

Kahn[11] corroborates this, getting complete anæsthesia of the skin in twenty-five minutes, by a current of 4·5 milliampères, with return of sensation in thirty minutes after cessation of current. With a current of 1 milliampère, the return of sensation was complete in ten minutes. An excised piece of skin which had been anæsthetised by passing 3·25 milliampères for thirty minutes through an anode filled with cocaine solution tinged with a blue dye stuff, on microscopic examination showed the dye stuff only to the depth of the rete Malpighii.

Lower mammals.—The results of observations upon absorption by the skin of lower mammals are here considered apart from those obtained from experiments on man, in order to obviate any tendency to treat the

[1] Bärensprung, *Journ. f. prakt. Chem.*, Leipzig, 1850, S. 50 ; Voit, *Ann. d. Chem.*, Leipzig, 1857, Bd. civ. S. 3 ; Hermann, "Lehrbuch d. exper. Toxicologie," Berlin, 1874, S. 212.

[2] *Loc. cit.* [3] Diss. Würzburg, 1854.

[4] *Arch. f. Dermat. u. Syph.*, Wien, Bd. iii. S. 309.

[5] Neumann, *Wien. med. Wchnschr.*, 1872.

[6] Porret, *Ann. d. Phys. u. Chem.*, Leipzig, Bd. lxvi. S. 272 ; du Bois-Reymond, *Monatsb. Akad. d. Wissensch.*, Berlin, 1860, S. 846 ; Wiedemann, *Ann. d. Phys. u. Chem.*, Leipzig, 1852, S. 321 ; and "Elektricitat," Braunschweig, 1883, Bd. ii. S. 166.

[7] Pascheles, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1895, Bd. xxxvi. S. 100.

[8] Engelmann, *Arch. neér. d. sc. exactes (etc.)*, 1874, Bd. ix. S. 332.

[9] Reichert, *Arch. f. Physiol.*, Leipzig, 1873, S. 505.

[10] *München. med. Wchnschr.*, Bd. xxxiii. S. 222.

[11] Inaug. Diss., Strassburg, 1891.

cases as analogous. The skin of the mammals usually employed for such experiments is thinner than that of man, less horny, more vascular on account of the hair, and in some cases (rabbit) possessed of hair follicles with wide mouths. The presence of hair is a source of trouble in experiment, for, if not shaved, excoriations may be passed over, while, on the other hand, the process of shaving is apt to be accompanied by slight injuries to the surface.

As with man, so here there is little positive evidence of absorption of watery solutions, and one is inclined to attribute the results of those observers who maintain that watery solutions are absorbed, to injuries produced in shaving, or clipping, or accidental introduction by mouth or lungs.

Forlanini[1] maintained that rabbits could be poisoned by painting aqueous solutions of strychnia, acidulated with acetic acid, on the skin, but v. Wittich[2] could not get the effect on white rats, nor Fubini and Pierini[3] with guinea-pigs, while Winternitz[4] obtained both positive and negative results with live rabbits. Fubini and Pierini allowed the tails of rats to soak in strychnia and potassium cyanide solutions (for forty minutes in the former case and two hours in the latter) without effect. Traube-Mengarini[5] painted the skin of dogs daily for two months with aqueous solution of potassium ferrocyanide, killed the animals, and treated skin sections with ferric chloride. The blue was only found between the surface cells, not reaching deeper than the stratum granulosum. Acidified borax-carmine solution, applied daily for seventy days, gave a like result. Fleischer[6] got iodine through the belly skin of a rabbit (into a watch-glass of water introduced under the skin) in two hours from a cylinder full of the tincture, but admits that the structure of the skin was altered.

With ether and chloroform solutions, absorption is more marked in the thin skin of the rabbit, guinea-pig, and rat, than in that of man.

Waller[7] immersed the leg of a guinea-pig in a mixture of chloroform and tincture of aconite, and was able to poison the animal, an effect not produced by the tincture alone. White rats with the foot in a chloroform solution of atropine, exhibited a dilated pupil in two or three minutes; with the tail (thicker skin) immersed, not till half an hour had elapsed. Strychnia in the same way he found was absorbed from solutions in chloroform, but not from those in alcohol.

Winternitz[8] also found that rabbits absorb strychnia solution in chloroform, and points out that this is not merely an effect of "stimulation," because a previous treatment of the skin with mustard or ammonia does not hasten the intoxication.

Winternitz has also pointed out that cleansing the skin of rabbits with ether or chloroform allows absorption of aqueous strychnia solution to take place, and, microscopically, it is found that silver nitrate solution penetrates more deeply if the skin is so treated. Alcoholic washing of the skin also tends to make subsequent absorption of aqueous solution possible, but to a far slighter degree than in the case of chloroform and ether.

[1] *Ann. univ. di med. e chir.*, Milano, 1868, vol. ccv. p. 473.
[2] *Loc. cit.* [3] *Loc. cit.* [4] *Loc. cit.*
[5] *Arch. f. Physiol.*, Leipzig, 1892, Supp., S. 1; *Arch. ital. de biol.*, Turin, 1891, vol. xvi. p. 159.
[6] *Loc. cit.* [7] *Proc. Roy. Soc. London*, 1860, vol. x. p. 122. [8] *Loc. cit.*

Experiments upon the absorption of oils and unguents by the skin of animals seem to have given conflicting results in the hands of different observers. Lassar[1] anointed rabbits with oil for days in succession, and maintains that the organs became loaded with oil. V. Sobieranski[2] asserts the same for vaseline rubbed into the skin of dogs and rabbits, and states that the substance is found especially in the muscles. Fleischer[3] denies the effect, as also does Winternitz,[4] though the latter observer was able to kill a rabbit by inunction of strychnia (2 per cent.) in oil. Adam and Schoumaker[5] got negative results from the inunction of an ointment of strychnia and vaseline into the skin of the necks of dogs. Mercury, however, is absorbed by dogs and horses from mercurial ointment. Thus Müller[6] rubbed mercurial ointment into clipped dogs and horses, and found mercury in the fæces and urine. An ointment of corrosive sublimate, sodic chloride, and fat, gave mercury in the fæces and urine; lead was passed after rubbing in an ointment of a lead salt, and application of a potassic iodide ointment gave iodine in the saliva. Aqueous solutions of sublimate were without effect when applied to the skin of these animals.

Cataphoric transfer of solutions through the skins of lower mammals can be induced more easily than in the case of man. Munk[7] was able to poison rabbits with strychnia in aqueous solution, and Kahn[8] obtained the pharmacological effects of physostigmine and strychnia on rabbits, and of apomorphine on dogs, by passing a current of 3·5 milliampères through ·2 per cent. solutions in the positive electrode, and in all cases proved that applications of the solutions without concomitant passage of current was without effect.

Frog.—In the case of the frog the conditions for absorption of watery solutions by the skin are far more favourable than in that of mammals, for the surface is kept constantly moist by the secretion of the skin glands, and no greasy matter is present, so that it is a matter of common laboratory experience that poisonous solutions applied to the skin of the animal rapidly produce their specific effects.

Blood vessels are abundant in the skin, especially in that of the back, and substances must diffuse with ease through or between the moist epidermic cells into the underlying vessels. It would, however, appear probable that, in addition to simple diffusion, the physiological condition of the lower epidermic cells affects the passage of substances through the skin.

Reid[9] found that the direction of easier osmotic transfer of fluid through freshly removed frog's skin is (provided the fluids used are not deleterious) from without inwards, *i.e.* the reverse of the direction of easier filtration through the dead skin; but that, as its vitality declines, the skin becomes less and less permeable from without inwards, and finally is more permeable in the reverse direction. The duration of the first period, during which the skin is more permeable

[1] *Virchow's Archiv*, 1879, Bd. lxxvii. S. 157; "Verhandl. d. physiol. Gesellsch.," in *Arch. f. Physiol.*, Leipzig, 1880, S. 563.
[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxxi. S. 329.
[3] *Virchow's Archiv*, Bd. lxxix. S. 558. [4] *Loc. cit.*
[5] *Journ. de pharmacol.*, Bruxelles, 1891.
[6] *Arch. f. wissensch. u. prakt. Thierh.*, Berlin, Bd. xvi. S. 309; reference in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1891, Bd. iv. S. 550.
[7] *Loc. cit.* [8] *Loc. cit.*
[9] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 132.

from without inwards, is directly associated with the vigour of the animals, lasting seventy to eighty hours after death in strong frogs, but only twenty-four hours or so in feeble animals at the end of the breeding season.

Again, the magnitude of an ordinary osmotic stream, maintained through freshly removed skin by means of solutions, whose injurious effect on tissue life is minimal, is capable of variation in the direction of increase or decrease, by such conditions as are known to exalt or depress the activity of living matter. If an osmotic current is set up in the direction from without inwards through living frog's skin (the normal direction of greater permeability when the skin is fresh), the presence of a stimulant (alcohol) increases, while that of a depressant (chloroform) decreases the current; on the other hand, if the osmotic current has been set up in the reverse direction, *i.e.* from within out, the stimulant causes diminution, and the depressant augmentation of the amount of fluid transferred from the inner to the outer surface of the skin in a given period of time. The phenomena failed to manifest themselves when dead skin was made the subject of experiment. The same observer [1] was also able to demonstrate the existence of a current of ·6 per cent. sodium chloride solution from the outer to the inner surface of freshly-removed skin, when the same solution at equal pressure was on either side, and hence filtration and osmosis put out of court.

These results are difficult to explain, and must provisionally be attributed to some unknown epithelial action.

[1] *Brit. Med. Journ.*, London, 13th Feb. 1892.

CHEMISTRY OF RESPIRATION.

By M. S. Pembrey.

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Respiration is essentially the intake of oxygen and the output of carbon dioxide by living cells. In the higher animals two phases of respiration are distinguished—the *external*, the exchange of gases between the air or water and the blood; and the *internal*, the exchange between the blood, lymph, and the tissues.

Historical Account.[1]—The view held by Aristotle (384–322 B.C.), and after him even until the fifteenth century, was that respiration drew air into the heart and arteries, and so cooled the blood. Malpighi (1621–1694) discovered the alveoli of the lungs, and saw the blood flowing through the capillaries of the alveoli of a frog's lung; and Fracassati,[2] in 1665, noticed that the lower layer of a blood clot was much darker in colour than the upper, but that on exposure to the air the lower became florid red. Hook[3] showed the following experiment at a meeting of the Royal Society in 1667. The ribs and diaphragm of a dog were cut away, and the trachea connected with a pair of bellows. The dog fell into convulsions, but revived when air was blown into the lungs. Numerous small holes were now made in the surface of the lungs, and by means of two bellows the lungs were kept constantly distended with fresh air; the dog lay still, and its heart beat regularly. A piece of lung was cut off, and it was noticed that the blood circulated even when the lungs were collapsed. Hook therefore came to the conclusion that the cause of death was not the stoppage of the circulation, but the want of a sufficient supply of fresh air. Croon[4] had previously shown before the same Society a similar experiment;

[1] For further details see Bostock's "Physiology," 2nd edition, 1828, vol. ii. p. 61; Paul Bert, "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 1; Zuntz, Hermann's "Handbuch," Bd. iv. Th. 2, S. 5.

[2] *Phil. Trans.*, London, 1667, p. 492.

[3] *Ibid.*, 1667, p. 539.

[4] Derham's "Physico-Theology," 4th edition, 1716, p. 146.

he strangled a pullet until it showed no signs of life, and then restored it by blowing air into its lungs.

Boyle,[1] in 1666, showed by numerous experiments with the air-pump that a supply of fresh air was essential to life, both animal and vegetable, and he was of the opinion "that the depuration of the blood was one of the ordinary and principal uses of respiration."

Mayow [2] (1668–1674) was the first to discover the real function of respiration; he showed that air was a mixture, and that one of its constituents, which he named the nitro-aerial gas, was necessary for the support of a flame, that it combined with sulphur and other substances with the production of acids, that during calcination metals also combined with it and thus increased in weight. The nitro-aerial gas (oxygen) was necessary for all forms of life, and the respiration of an embryo was analogous to that of the adult. Mayow saw the analogy of respiration to combustion, and held that the function of respiration was to absorb the nitro-aerial gas and to remove the vapours arising from the blood.

Stephen Hales,[3] about the year 1726, showed that animals in a closed vessel absorb air, and that a similar change is effected by a burning candle. He also observed, by experiments upon himself, that air is absorbed during respiration, and that "noxious vapours" are produced by repeatedly breathing air in a bladder; these noxious substances, he found, could be removed by potash, and the air rendered fit for breathing. Hales suggested the use of a bladder of air and such an absorbent in the foul air of coal mines. He believed that during respiration the air cooled the blood and removed aqueous vapour and noxious substances, but he rejected the view of Mayow that the blood combined with the nitro-aerial gas.

About the year 1757, Black [4] discovered that a quantity of "fixed air" (carbon dioxide) was given off from the lungs, and that the expired air chiefly differed from the inspired by the addition of that gas. He observed that animals placed in carbon dioxide gas died of suffocation.

In 1772, Priestley [5] published his "Observations on Different Kinds of Air," in which he showed that growing plants restored the property of supporting animal life to air which had been vitiated by the respiration of animals or by the burning of a candle. He also found that carbon dioxide was produced by putrefaction and by plants during the night-time. Priestley isolated oxygen and nitrogen, and showed that the change of colour in venous blood on exposure to the air was due to the action of oxygen, and that blood changed colour and gave off "phlogiston" even when it was separated from the air by a moist membrane and by the walls of the blood vessels in the lungs. He concluded that respiration deprived the air of a portion of its oxygen and imparted to it a quantity of aqueous vapour and "phlogiston."

Lavoisier [6] (1777) extended and explained the discoveries of Mayow, Black, and Priestley; he overthrew the old theory of "phlogiston," and pointed out a distinction between the various so-called phlogistic processes. The calcination of metals he showed, as Mayow had observed a hundred years before, to be a combination with oxygen, whereby the metals gained in weight; in respiration, on the other hand, oxygen was not only absorbed, but combined with carbon to form carbon dioxide.

Lavoisier and Laplace showed experimentally that animal heat arose from a process of combustion, oxygen combining, as they thought, with carbon in the blood; as regards the seat of this combustion, Lavoisier held that it was

[1] *Phil. Trans.*, London, 1666, p. 424; 1670, pp. 2011, 2035.
[2] *Ibid.*, 1668, p. 833; "Tractatus quinque," Oxon. 1674.
[3] "Statical Essays," 2nd edition, 1731, vol. i. p. 236 *et seq.*
[4] "Lectures on Chemistry," ed. Robison, Edinburgh, 1803.
[5] *Phil. Trans.*, London, 1772, vol. lxii., p. 147.
[6] *Hist. Acad. roy. d. sc.*, Paris, 1775, 1777, 1780, 1789, and 1790.

in the lungs, but in earlier works he had admitted that it might be in the other organs of the body.[1]

It is now known that the essential seat of respiration is in the tissues and not in the blood. The demonstration of this fact is chiefly due to the work of Pflüger and his pupils.

Respiratory Changes in Air.

Methods for the measurement of respiratory exchange.—The simplest and at the same time the earliest method for the measurement of respiratory exchange, is the analysis of the air of a bell jar, before and after an animal has been confined in it. Such a method was used by Black,[2] Priestley,[3] Lavoisier and Laplace,[4] and others.[5] The obvious objection to this method is that the products of respiratory exchange

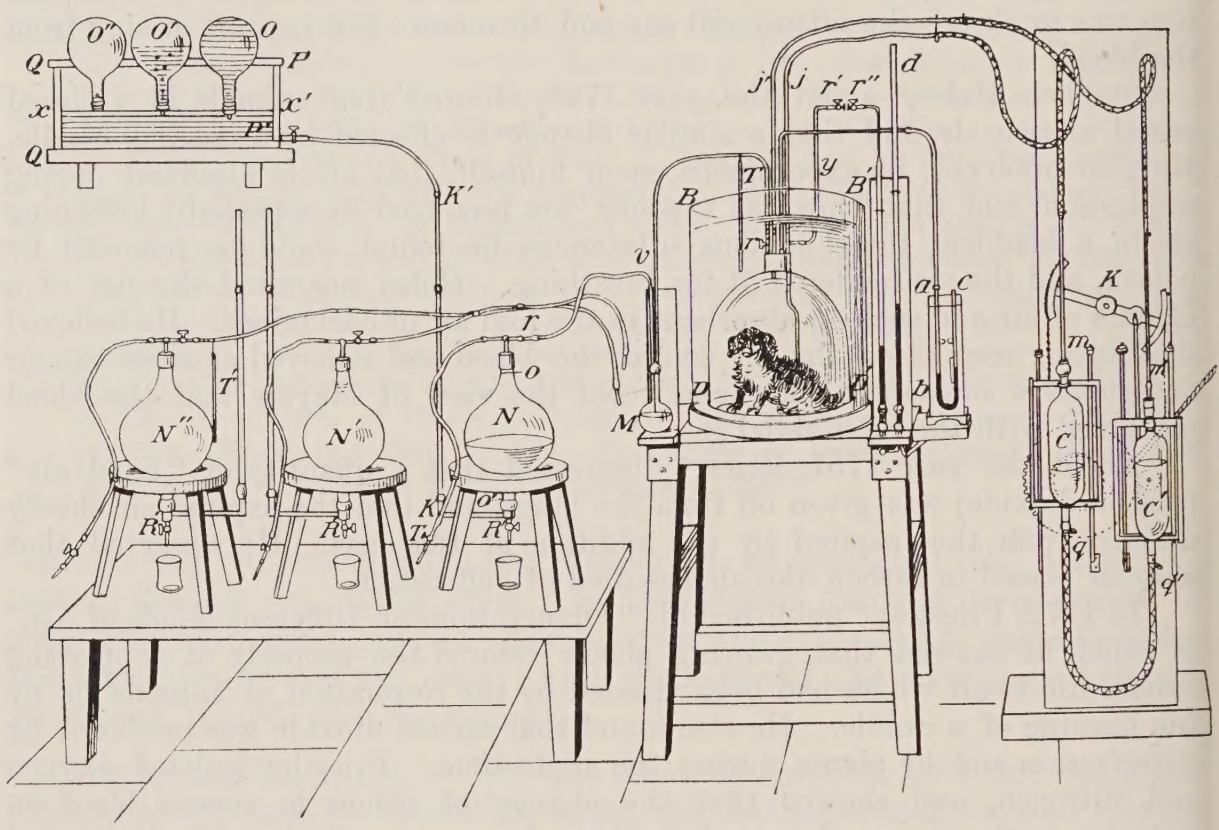


Fig. 62.—Regnault and Reiset's respiration apparatus.

accumulate, while the oxygen diminishes, two conditions either of which disturbs the normal respiratory exchange, and in time causes the death of the animal.[6] Two modifications were introduced by Lavoisier to remove these defects: in the one, the carbon dioxide was removed as it accumulated, and a fresh supply of oxygen was added; in the other, a constant stream of fresh air was passed through the respiration chamber.

Upon the first of these principles, Regnault and Reiset[7] constructed the apparatus with which they made numerous and important experiments upon respiratory exchange. The above figure shows its construction.

[1] "Œuvres," 1862, p. 180. [2] "Lectures on Chemistry," ed. Robison, Edinburgh, 1803.
[3] *Phil. Trans.*, London, 1772, vol. lxii. pp. 147, 168.
[4] *Hist. Acad. roy. d. sc.*, Paris, 1780, p. 355.; "Œuvres de Lavoisier," tome ii. p. 326.
[5] Berthollet, *Journ. f. Chem. Physik. u. Min.*, Berlin, 1808, Bd. v. S. 388; Legallois, *Journ. f. Chem. u. Phys.*, Nürnberg, 1817, Bd. xx. S. 113; Valentin, "Die Einflusse der Vaguslähmung auf die Lungen und Hautausdünstung," Frankfurt a/M., 1857; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1876, Bd. v. S. 143.
[6] Bernard, "Leçons sur les effets des substances toxiques," 1857, p. 130; Friedländer and Herter, *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 19; Stroganow, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 18. See also this article, p. 743.
[7] *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi.

The carbon dioxide is absorbed from the air by caustic potash, and a constant supply of oxygen from the reservoirs is driven in, a manometer in communication with the animal chamber indicating the pressure. Samples of air for analysis can be drawn from the chamber, and thus the part played by nitrogen determined, and a control placed upon the completeness of the supply of oxygen and the removal of carbon dioxide. Modified forms of Regnault and Reiset's apparatus have been used by Hoppe-Seyler and Stroganow,[1] Pflüger and Colasanti,[2] Schulz,[3] Seegen and Nowak.[4]

In Scharling's[5] respiration apparatus a constant stream of fresh air was drawn through the chamber in which the animal was confined. A big barrel served for the chamber, and air freed from carbon dioxide by passing through Liebig's potash bulbs was aspirated through the apparatus; on leaving the chamber the air passed through a flask containing sulphuric acid, which removed the moisture, and through a weighed potash bulb of huge size, of which the increase in weight gave the amount of carbon dioxide expired by the animal. As a control, a sample of air for analysis was removed from the barrel at the beginning and end of the experiment. In this method the carbon dioxide alone was determined, and the results were inaccurate, for the absorption was incomplete, as is shown by the fact that the air leaving the bulbs rendered lime water turbid. Many other forms of apparatus constructed upon similar principles have been used.[6]

With the methods formerly in use it was impossible to maintain a steady ventilation, and at the same time completely absorb the carbon dioxide. To overcome this difficulty, Pettenkofer[7] introduced the following modification. The total amount of air drawn through the apparatus is measured by a meter; continuous samples of the air entering and leaving the chamber are steadily drawn through two separate systems of absorption tubes and meters for the determination of the moisture, carbon dioxide, and volume of the samples. The difference in the amounts of water and carbon dioxide contained in the two samples, multiplied by the total ventilation, gives the quantity of moisture and carbon dioxide discharged by the animal. The intake of oxygen is estimated in the following way. The animal is weighed at the beginning and at the end of the experiment, and the difference between the weights of carbon dioxide and water discharged, and the loss in weight of the animal, represents the oxygen absorbed. Thus if W represents the initial weight of the animal, and W_1 its final weight, then $W - W_1 = w$, the loss in weight of the animal. Let $CO_2 + H_2O$ represent the weights of carbon dioxide and water discharged during the experiment, then $CO_2 + H_2O - w = O_2$, the oxygen absorbed. In thus estimating the oxygen, it is assumed that, apart from the carbon dioxide and

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 18.
[2] *Ibid.*, 1877, Bd. xiv. S. 92.
[3] *Ibid.*, Bd. xiv. S. 78.
[4] *Ibid.*, 1879, Bd. xix. S. 347.
[5] *Ann. d. Chem. u. Pharm.*, 1843, Bd. xlv. S. 214.
[6] Allen and Pepys, *Phil. Trans.*, London, 1809, pt. 2, p. 412; Dulong, *Ann. de chim. et phys.*, Paris, 1841, Sér. 3, tome i. p. 440; Despretz, *ibid.*, 1824, tome xxvi. p. 337; Boussingault, *ibid.*, 1844, Sér. 3, tome xi. p. 433; *Journ. f. prakt. Chem.*, Leipzig, 1845, Bd. xxxv. S. 402; Senator, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1872, S. 1; Liebermeister, *Deutsches Arch. f. klin. Med.*, Leipzig, 1870, Bd. vii. S. 75.
[7] *Sitzungsb. d. k.-bayer. Akad. d. Wissensch. zu München, math.-phys. Cl.*, 1862, Bd. ix. (2), S. 232; *Ann. d. Chem. u. Pharm.*, 1862–63, Supp. Bd. ii. S. 17.

water, no weighable amount of nitrogen, or of any other gaseous substance, is discharged or absorbed by the animal.

The figure below represents the modification of Pettenkofer's apparatus, which was introduced by Voit for experiments on animals.

The absorption of water is effected by flasks filled with pieces of pumice saturated with sulphuric acid, and the carbon dioxide is in turn absorbed by making the air bubble through a long tube filled with a titrated solution of baryta.[1]

The advantages of Pettenkofer's method over those previously in use are these—It is possible, owing to the system of ventilation, to make experiments upon man; observations can for a similar reason be much more prolonged without any danger of disturbance to the normal respiratory exchange arising from an accumulation of carbon dioxide; the absorption of the carbon dioxide is more exact. Notwithstanding these improvements, Pettenkofer's method possesses several disadvantages and sources of error. The apparatus is complicated and costly, the

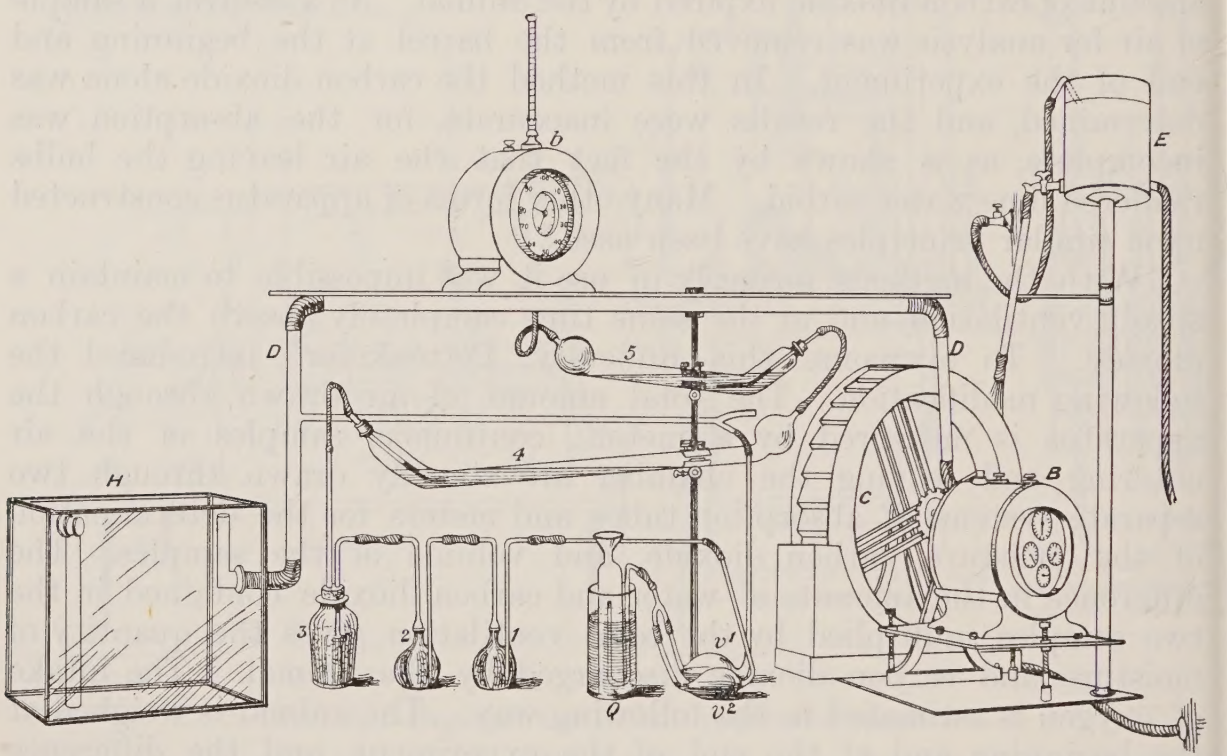


FIG. 63.—Voit's respiration apparatus.

determination of the moisture is liable to be inexact, owing to deposition on the walls of the chamber; during the process of weighing the animal there is an intake of oxygen, and an output of carbon dioxide and water, which are not determined, and can only be calculated approximately; the absorption and estimation of carbon dioxide by the titration of the baryta solution has been shown by Haldane and Pembrey[2] to be less exact than it was thought to be. The result of these errors falls upon the estimation of the intake of oxygen, for since $O_2 = CO_2 + H_2O - w$, it is evident that the amount of oxygen may be often inexact. This has been pointed out and proved by C. and E. Voit and Forster.[3]

It has already been mentioned that Voit[4] has constructed, upon Pettenkofer's principle, a smaller apparatus for the determination of

[1] Baryta was first used by Pettenkofer for this purpose, but Dalton had previously used titrated lime water.

[2] *London, Edinburgh, and Dublin Phil. Mag.*, London, April 1890.

[3] *Ztschr. f. Biol.*, München, 1875, Bd. xi. S. 126. [4] *Ibid.*, 1878, Bd. xiv. S. 57.

the respiratory exchange in animals. It has the advantages and most of the disadvantages above mentioned. The human respiration apparatus in the physiological laboratory, Oxford, has been constructed on the

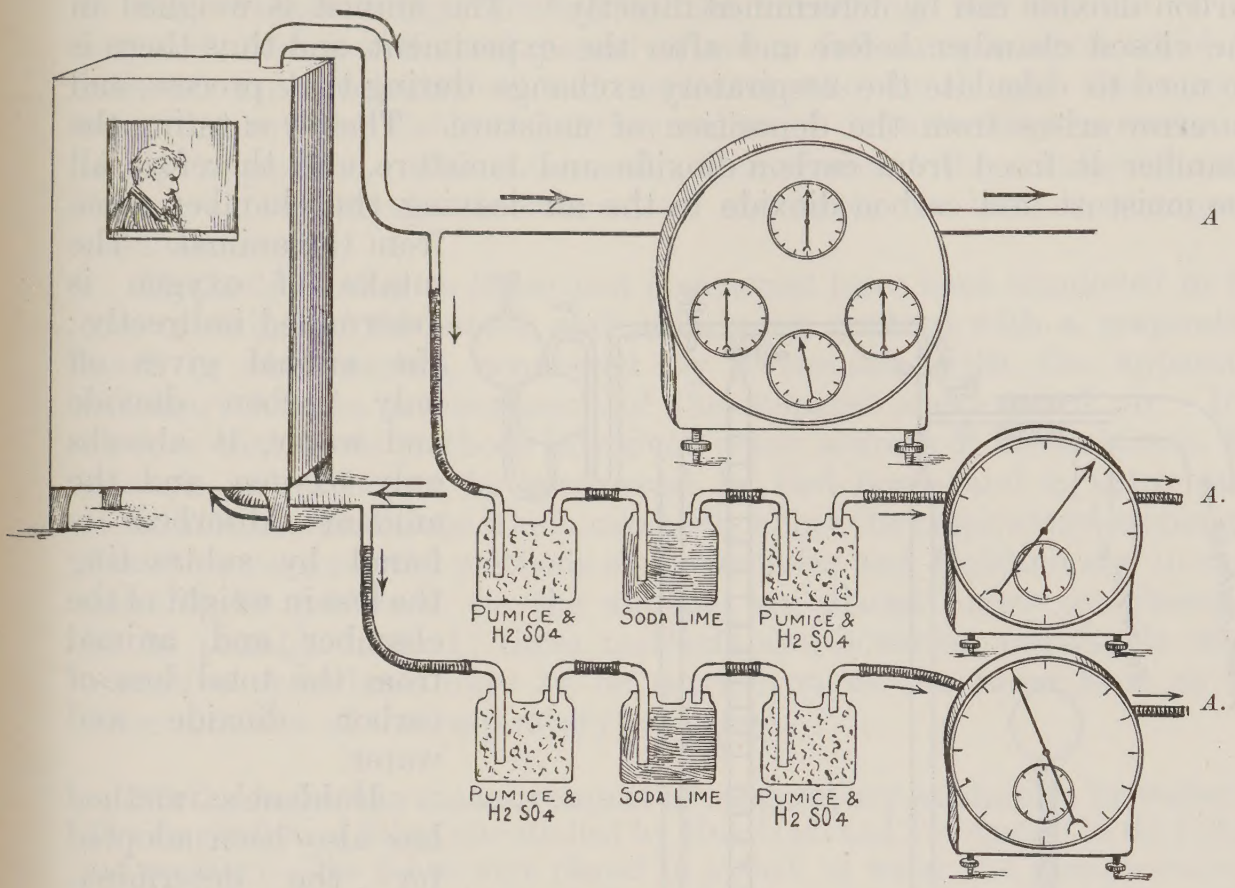


FIG. 64.—Diagram of the human respiration apparatus in the Physiological Laboratory Oxford.—*A.* To Aspirator.

principle of Pettenkofer's apparatus, but has been made more exact and simple by the use of Haldane and Pembrey's method of determining carbon dioxide and moisture.

A more exact method is that introduced by Haldane.[1] It is a

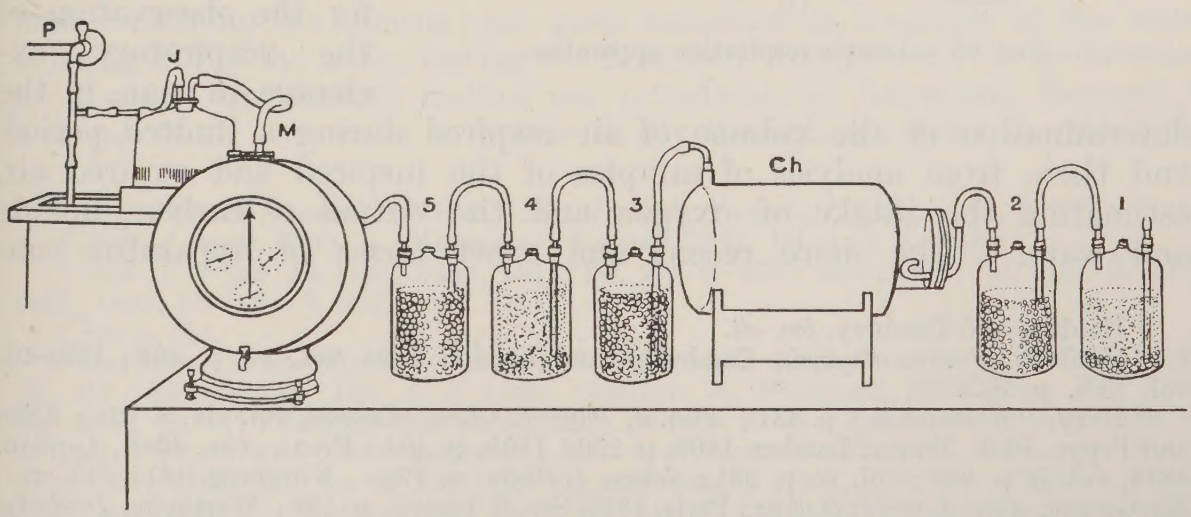


FIG. 65.—Haldane's respiration apparatus.—1 and 4, soda lime; 2, 3, and 5, pumice soaked in sulphuric acid; Ch, chamber for animal; M, gasmeter; J, water manometer; P, aspirator.

modification of the apparatus used by Scharling and Pettenkofer, but the chief sources of error have been eliminated or greatly diminished, and the method has been made extremely simple. The construction is shown in Fig. 65 :—

[1] *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 419.

The moisture is absorbed by pumice saturated with sulphuric acid, and the carbon dioxide is removed by soda lime, which has been proved to be such a rapid and excellent absorbent that the total output of carbon dioxide can be determined directly.[1] The animal is weighed in the closed chamber before and after the experiment, and thus there is no need to calculate the respiratory exchange during that process, and no error arises from the deposition of moisture. The air entering the chamber is freed from carbon dioxide and moisture, and therefore all the moisture and carbon dioxide in the air leaving the chamber come from the animal. The intake of oxygen is determined indirectly; the animal gives off only carbon dioxide and water, it absorbs only oxygen, and the amount absorbed is found by subtracting the loss in weight of the chamber and animal from the total loss of carbon dioxide and water.

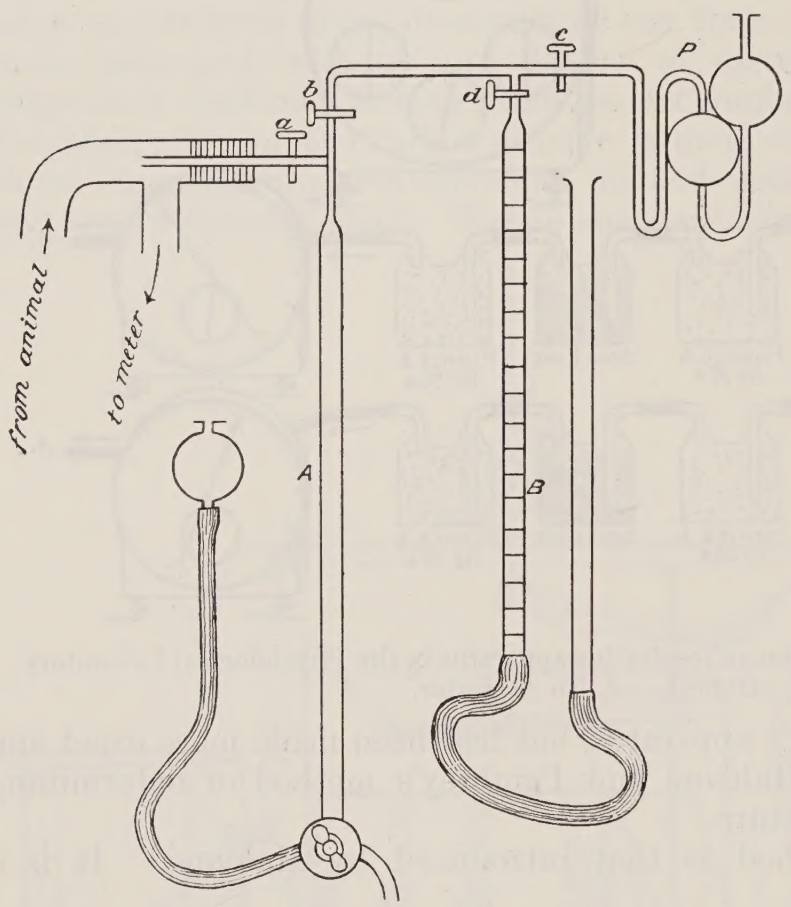


FIG. 66.—Löwy's respiration apparatus.

Haldane's method has also been adopted for the determination of the respiratory exchange of small animals and of chick embryos.[2]

Another method, which has been used for the observation of the respiratory exchange in man, is the determination of the volume of air respired during a limited period, and then, from analysis of samples of the inspired and expired air, estimating the intake of oxygen and the output of carbon dioxide and water.[3] The more recent and exact forms of apparatus con-

[1] Haldane and Pembrey, *loc. cit.*

[2] Pembrey, *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 401; 1894–95, vol. xvii. p. 331.

[3] Davy, "Researches," p. 431; *Ann. d. Phys. u. Chem.*, Leipzig, Bd. xix. S. 298; Allen and Pepys, *Phil. Trans.*, London, 1808, p. 250; 1809, p. 404; Prout, *Ann. Phil.*, London, 1813, vol. ii. p. 330; vol. iv. p. 331; *Journ. f. Chem. u. Phys.*, Nürnberg, 1814, Bd. xv.; MacGregor, *Ann. de chim. et phys.*, Paris, 1841, Sér. 3. tome ii. p. 538; Wertheim, *Deutsches Arch. f. klin. Med.*, Leipzig, Bd. xv.; *Wien. med. Wchnschr.*, 1878; Vierordt, "Physiol. d. Athmens," Karlsruhe, 1845; E. Smith, *Phil. Trans.*, London, 1859, vol. cxlix. p. 682; Speck, "Untersuch. ueber Sauerstoffverbrauch u. Kohlensäureausathmung d. Menschen," Cassel, 1871; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. ii. S. 405; Bd. xii. S. 1; Lossen, *Ztschr. f. Biol.*, München, 1866, Bd. ii. S. 244; Berg, *Deutsches Arch. f. klin. Med.*, Leipzig, 1869, Bd. vi. S. 291; Leyden, *ibid.*, Bd. vii. S. 536; Andral and Gavarret, "Recherches sur l'acide carbonique exhalé," Paris, 1843; Marcet, *Phil. Trans.*, London, 1890, B.; *Proc. Roy. Soc. London*, 1891, vol. xlix. p. 103; Jolyet, Bergonié, and Sigalas, *Compt. rend. Acad. d. sc.*, Paris, 1887, tome cv. p. 380.

structed upon these principles are those used by Zuntz,[1] Geppert[2] and Löwy.[3] A diagram of such an apparatus is shown in Fig. 66.

The disadvantage of these methods is that, owing to the attention of the subject being directed to the breathing, the volume of air respired during a limited period is not a fair sample upon which to base an exact calculation, and, moreover, the depth and the rate of breathing are also liable to another source of disturbance, the resistance of the apparatus. For these and other reasons,[4] the results obtained during short periods of observation are liable to lead to erroneous conclusions.

Methods similar to those just mentioned have been employed in the case of animals,[5] the mouth and nose being covered with a respiration mask or the trachea connected by a cannula with the apparatus necessary for the measurement of the inspired and expired air. It is obvious that these methods introduce many sources of disturbance; the animals, unless horses be used, must be tied down, and in many cases anæsthetised, conditions which markedly affect the respiratory exchange.[6] For these reasons the methods of Pettenkofer and Haldane are in most cases to be preferred, for the animals are placed under conditions as far as possible normal; these methods are, however, unsuitable when operative procedures have to be carried on at the same time as the determination of the respiratory exchange.

Methods for the measurement of respiratory exchange in water.—The respiration of fishes was studied by Humboldt and Provençal[7] in the following manner:—The fishes were placed in a flask of water, the gaseous contents of which had been analysed, and then after an interval a sample of the water was examined and the alteration in its gases determined. The quantity of water present was measured, and thus it was possible to estimate the amount of gases absorbed and discharged by the fish. A similar method has been used by Vernon[8] for the measurement of the respiratory exchange in marine invertebrates.

Baumert[9] improved this method by passing a stream of water through the flask containing the animals; the gases contained in a sample of the water entering and in the water leaving the flask were determined. A modification of Regnault and Reiset's method was introduced by Jolyet and Reynard;[10] a stream of air was made to bubble slowly through the water in which the

[1] *Berl. klin. Wchnschr.*, 1887, S. 429; *Arch. f. Physiol.*, Leipzig, 1889, S. 166.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 368.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 268; *ibid.*, 1888, Bd. xliii. S. 519; *ibid.*, 1891, Bd. xlix. S. 492.

[4] See p. 754.

[5] Sczelkow, *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1862, Bd. xlv; Kowalewsky, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Kl.*, 1866, Bd. xviii. S. 111; Sanders-Ezn, *ibid.*, 1867, Bd. xix. S. 58; *Arb. a. d. physiol. Anst. zu Leipzig*, 1868, S. 58; Scheremetjewski, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Kl.*, 1868, Bd. xx. S. 154; Röhrig and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 57; Zuntz, *ibid.*, 1876, Bd. xii. S. 522; Finkler and Oertmann, *ibid.*, 1877, Bd. xiv. S. 38; Pflüger, *ibid.*, 1878, Bd. xviii. S. 247; Hanriot and Richet, *Compt. rend. Soc. de biol.*, Paris, 1886; *Compt. rend. Acad. d. sc.*, Paris, 1887, tome civ. p. 435; Fredericq, *Rev. scient.*, Paris, 1880; *Bull. Acad. roy. d. sc. de Belg.*, Bruxelles, 1886.

[6] See p. 717. See also "Animal Heat," this Text-book, vol. i.

[7] *Mém. de la Soc. de phys. et de chim. d'Arcueil*, Paris, 1807, tome ii. p. 359; *Journ. f. Chem. u. Phys.*, Nürnberg, Bd. i. S. 86.

[8] *Journ. Physiol.*, Cambridge and London, 1895-96, vol. xix. p. 18.

[9] "Chem. Untersuch. u. d. Respir. d. Schlammpeitzgers," Breslau, 1855, S. 24.

[10] *Arch. de physiol. norm. et path.*, Paris, 1877, tome iv. p. 44.

animal was placed; this air was analysed for carbon dioxide, and the oxygen absorbed by the animal was replaced by a corresponding amount supplied from a gasometer.

The consumption of oxygen by animals living in water can be determined by titrating a sample of the water before and after the confinement of the animal in a known volume of water. Quinquand[1] used for this purpose sodium hyposulphite, according to Schützenberger's method.

The conditions which affect the respiratory exchange. -- A determination of the respiratory exchange not only gives the absolute value of the oxygen absorbed, and of the carbon dioxide and water excreted, but also shows the relationship between the intake of oxygen and the output of carbon dioxide. This ratio between the volume of oxygen absorbed and the volume of carbon dioxide discharged is known as the *respiratory quotient* $\frac{CO_2}{O_2}$, and indicates how much of the oxygen combines with carbon to form carbon dioxide, for one volume of oxygen in combining with carbon yields one volume of carbon dioxide. Various conditions influence both the amount of the respiratory exchange and the relative proportions of the gases, but it must be remembered that determinations of short duration may give rise to erroneous conclusions, for oxygen may be stored up for some time within the body, and carbon dioxide may still be formed and discharged when there is no intake of oxygen.

The question here arises, Does nitrogen play any active part in respiration, is there any absorption or discharge of nitrogen?[2] The older observers found that nitrogen was sometimes absorbed by the lungs, and in nearly all of Regnault and Reiset's[3] determinations of the respiratory exchange in different animals there is an alteration in the amount of nitrogen present in the air, denoting generally a discharge of a small quantity of nitrogen from the animal. Marchand[4] had also obtained similar results; he found in ten experiments upon guinea-pigs that the average discharge of nitrogen was equal to 0·94 per cent. of the output of carbon dioxide, and in three experiments on pigeons to 0·85 per cent. Seegen and Nowak[5] also found a discharge of nitrogen, varying from 4 to 9 mgrms. per kilo. and hour in thirty-two experiments upon rabbits, dogs, and hens.

This discharge of nitrogen in many cases appears to be due to an error of experiment.[6] Analyses, purposely made by Colasanti[7] to test this point, showed no discharge or absorption of nitrogen by guinea-pigs. The small amount observed by other experimenters may be due either to nitrogen discharged from the alimentary canal or to experimental errors. According to Jolyet, Bergonié, and Sigalas,[8] an amount of nitrogen varying from $\frac{2}{100}$ to $\frac{8}{1000}$ of the oxygen absorbed is taken up by the blood in its passage through the lungs of a man or a dog. In any case the amount of nitrogen absorbed or discharged under

[1] *Compt. rend. Acad. d. sc.*, Paris, 1873, tome lxxvi. p. 1141.
[2] For further details, see Voit, Hermann's "Handbuch," Bd. vi., Th. 1, S. 37.
[3] *Ann. de chim. et phys*, Paris, 1849, Sér. 3, tome xxvi.
[4] *Journ. f. prakt. Chem.*, Leipzig, 1848, Bd. xliv. S. 1.
[5] *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1875, Bd. lxxi. (3), S. 329 ; *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix. S. 347.
[6] Pettenkofer and Voit, *Ztschr. f. Biol.*, München, 1880, Bd. xvi. S. 508.
[7] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 92.
[8] *Compt. rend. Acad. d. sc.*, Paris, 1887, tome cv. p. 675.

ordinary conditions is so small that it may be neglected. It is to be noted, however, that Colasanti and Finkler[1] always found small quantities of marsh gas and hydrogen in the respiration chamber in which well-fed guinea-pigs were placed; these gases probably came from the alimentary canal, for they were not found in the case of guinea-pigs deprived of food. Zuntz[2] and Tacke found that three-quarters of the hydrogen and marsh gas formed in the alimentary canal of a rabbit were absorbed by the blood and discharged by the lungs.

The respiratory exchange of cold-blooded animals. — When compared with warm-blooded animals, the respiratory exchange of most cold-blooded animals is very small, a fact which explains the small production of heat observed in this class of animals.[3] Some of the earliest determinations were those made by Vauquelin,[4] Spallanzani,[5] Newport,[6] Treviranus,[7] Edwards,[8] and Müller.[9] They showed that the quantity of oxygen consumed and of carbon dioxide produced was for equal weights of animals generally much less in cold-blooded than in warm-blooded animals, the most marked exception being in insects. Later researches have confirmed these general conclusions, and have shown the conditions which chiefly affect the respiratory exchange in these animals. Of these conditions the most important is the external temperature, a rise in temperature causing an increase, a fall in temperature a decrease in the respiratory exchange. In the following table the results of various observers are expressed for 1 kilo. weight of animal and 1 hour, in order that they may be comparable:—

Animal.	Weight (in Grms.).	Oxygen per Kilo and Hour (in Grms.)	Carbon Dioxide per Kilo and Hour (in Grms.)	$\frac{CO_2}{O_2}$	Temperature.	Remarks.	Observer.
PROTOZOA—							
Collozoum inerme.	..	..	·1113	1·06	16°	One determination.	Vernon.[10]
CŒLENTERATA—							
Carmarina hastata	..	..	·0087	1·10?	16°	The determinations of $\frac{CO_2}{O_2}$ show variations from ·36 to 2·16.	,,
Cestus veneris .	..	..	·0037	·79?	16°	$\frac{CO_2}{O_2}$ varies from ·07 to 2·06.	,,

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xv. S. 603.
[2] *Arch. f. Physiol.*, Leipzig, 1894, S. 354. See also this article, p. 729.
[3] Article "Animal Heat," this Text-book, vol. i. p. 792.
[4] *Ann. de. chim.*, Paris, 1792, tome xii. p. 273.
[5] "Mém. sur la respiration," par Senebier, 1803, p. 184; *Journ. f. Chem. Physik. u. Min.*, Berlin, Bd. iii. S. 378.
[6] *Phil. Trans.*, London, 1837, pt. ii. p. 253.
[7] *Ztschr. f. Physiol.*, 1832, Bd. iv. S. 23.
[8] "De l'influence des agens physiques sur la vie," Paris, 1824.
[9] "Elements of Physiology," trans. Baly, 1838, vol. i. p. 310.
[10] *Journ. Physiol.*, Cambridge and London, 1895-96, vol. xix. p. 18.

TABLE—*continued.*

Animal.	Weight. (in Grms.).	Oxygen per Kilo and Hour (in Grms.).	Carbon-Dioxide per Kilo. and Hour (in Grms.).	$\frac{CO_2}{O}$	Temperature.	Remarks.	Observer.
VERMES [1]—							
Lumbricus . .	112	0·1013 (70·8 c.c.)	0·108 (54·9 c.c.)	·77	?	.	Regnault and Reiset. [2]
,,	..	..	0·593	..	18°–19°	One determination.	Pott. [3]
Hirudo . .	..	..	0·645 (328 c.c.)	..	16°–19°	Mean of two determinations.	,,
Hirudo officinalis	2·2	0·0328 (22·98 c.c.)	0·0312 (15·9 c.c.)	·69	13°·5	Before } a meal.	Jolyet and Regnard. [4]
,, ,,		0·0567 (39·70 c.c.)	0·0701 (35·7 c.c.)	·90	13°	After } a meal.	,,
INSECTA—							
Anthercea (18) .	42·5	0·840 (588 c.c.)	0·916 (465·7 c.c.)	·79	?	..	Regnault and Reiset.
,, ,,	39	0·687 (480 c.c.)	0·767 (390·7 c.c.)	·81	?	..	,,
,, 42)	40	1·170 (818 c.c.)	1·189 (604·5 c.c.)	·73	?	..	,,
Melolontha (40)	40·3	1·076 (752 c.c.)	1·1699 (594·8 c.c.)	·79	?	..	,,
,, (37)	37	0·962 (673 c.c.)	1·092 (555 c.c.)	·82	?	..	,,
Geotrupes . .	0·32	..	1·130 (574 c.c.)	..	20°–21°	Mean of two determinations.	Pott.
Melolontha . .	2·0	..	0·987 (503 c.c.)		15°–17°	Larva. Mean of four determinations.	,,
Locusta . .	..	..	0·839 (427 c.c.)	..	16°–19°	Mean of three determinations.	,,
Gryllus campestris	0·25	..	2·305 (1172 c.c.)	..	..	,, ,,	,,
,, ,,	0·05	..	2·127 (1081 c.c.)	..	17°–21°	Mean of two determinations.	,,
Blatta orientalis .	..	..	0·268	..	15°–20°	..	Bütschli. [5]
,, ,,	..	..	1·045	..	30°–35°	..	,,
MOLLUSCA—							
Octopus vulgaris	2310	·0630 (44·1 c.c.)	·0745 (37·9 c.c.)	·86	15°·5	..	Jolyet and Regnard.
,, ,,	..	..	·1115	·95	16°	..	Vernon.
Tethys leporina .	..	..	·0165	·84	16°	..	,,
CRUSTACEA—							
Astacus fluvialis .	31	·0543 (38 c.c.)	·0642 (32·7 c.c.)	·86	12°·5	..	Jolyet and Regnard.
Gammarus pulex	..	(132 c.c.)	(95 c.c.)	·72	12°·5	..	,,
Palemon squilla .	..	(125 c.c.)	(103·8 c.c.)	·83	19°	..	,,
Cancer pagurus .	470	(107 c.c.)	(89·9 c.c.)	·84	16°	..	,
Homarus vulgaris	315	(68 c.c.)	(54·4 c.c.)	·80	15°	..	
Palinurus quadricorn	520	(44·2 c.c.)	(38·9 c.c.)	·88	15°	..	

[1] For experiments upon the respiration of nematoid worms, see Bunge, *Ztschr. f. physiol. Chem.*, Strassburg, 1883, Bd. viii. S. 48 ; 1890, Bd. xiv. S. 318.

[2] *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi.

[3] *Landwirthsch. Versuchsstat.*, Bd. xviii. S. 81.

[4] *Arch. de physiol. norm. et path.*, Paris, 1877, tome iv. p. 44.

[5] Bütschli, *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1874, S. 348. For further details on the respiratory exchange of insects, see Spallanzani, *Journ. f. Chem. Physik. u. Min.*, Berlin, Bd. iii. S. 378 ; Vauquelin, *Ann. de chim.*, Paris, 1792, tome xii. ; Treviranus, *Ztschr. f. Physiol.*, 1832, Bd. iv. S. 23 ; Newport, *Phil. Trans.*, London, 1837, pt. ii. p. 259 ; Detmer, *Landwirthsch. Versuchsstat.*, Bd. xv. S. 196 ; Liebe, *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, 1872, S. 332 ; Schmidt-Schwedt, *Berl. entomol. Ztschr.*, Bd. xxxi. S. 325.

TABLE—*continued.*

Animal	Weight (in Grms.).	Oxygen per Kilo and Hour (in Grms.).	Carbon-Dioxide per Kilo and Hour (in Grms.).	$\frac{CO_2}{O_2}$	Temperature.	Remarks.	Observer.
PISCES—							
Cyprinus auratus	12–14	·0376 (26·3 c.c.)	·0411 (20·9 c.c.)	·79	8°	Fasting.	Baumert.[1]
,, ,,	12–14	·0590 (41·3 c.c.)	·0617 (31·4 c.c.)	·76	11°–12°	Fasting. Mean of three determinations.	,,
,, ,,	33–40	·0655 (45·8 c.c.)	·0643 (32·7 c.c.)	·71	12°	Mean of two determinations.	Jolyet and Regnard.
Cyprinus tinca .	222	·0796 (55·7 c.c.)	·0721 (36·7 c.c.)	·66	14°	..	..
,, ,,	500	·1001 (70·0 c.c.)	..	..	?	..	Quinquand.[2]
,, ,,	190-223	·0165 (11·53 c.c.)	·0159 (8·09 c.c.)	·70	5°	..	Baumert.
Cyprinus carpio .	12	..	0·352 (179 c.c.)	..	13°–14°	Young. Mean of two determinations.	Pott.
Cobitis fossilis .	43–61	·0455 (31·8 c.c.)	·0633 (32·2 c.c.)	1·01	9°–12°	Mean of six determinations.	Baumert.
,, ,,	16	·1234 (86·3 c.c.)	·1323 (67·3 c.c.)	·78	17°–22°	..	Jolyet and Regnard.
Murœna anguilla	51	·0580 (40·5 c.c.)	·0629 (32 c.c.)	·79	14°	..	,,
,, ,,	112	·0686 (48·0 c.c.)	·0566 (28·8 c.c.)	·60	15°	..	,,
Mullus . .	28	·1916 (134 c.c.)	·2132 (108·5 c.c.)	·81	14°	..	,,
Sparus auratus .	75	·2031 (142 c.c.)	·1787 (90·9 c.c.)	·64	19°	..	,,
Trigla hirundo .	350	·1351 (94·5 c.c.)	·1337 (68 c.c.)	·71	15°	..	,,
Murœna conger .	545	·0855 (59·8 c.c)	·0865 (43 c.c.)	·72	13°	..	,,
Raja torpedo .	315	·0672 (47·0 c.c.)	·0540 (27·5 c.c.)	·58	14·5°	Mean of two determinations.	,,
AMPHIBIA—							
Axolotl . .	42	·0646 (45·2 c.c.)	·0497 (25·3 c.c.)	·56	11°·5	..	Jolyet and Regnard.
Rana esculenta .	..	·063 (44·2 c.c.)	0·060 (30·76 c.c.)	·69	15°–19°	Minimal and maximal of five experiments.	Regnault and Reiset.
,, ,,	..	·105 (73·4 c.c.)	0·1134 (57·7 c.c.)	·78	..	Minimal and maximal of five experiments.	Regnault and Reiset.
,, ,,	..	(50·46 c.c.)	(47·98 c.c.)	·95	17°·7	..	Oertmann.
,, ,,	..	..	0·082	..	17°	..	Schulz.[3]
,, *temporaria*	13·9	..	0·355	..	19°–20°	..	Pott.
,, ,,	..	..	0·502	..	23°·1	Mean of twenty-two experiments.	Moleschott.
,, ,,	31·64	..	0·038	..	13°	Duration of experiment was three and four hours (winter frog).	Pembrey.
,, ,,	31·64	..	0·035	..	13°	Duration of experiment was three and four hours (winter frog).	Pembrey.
,, ,,	34·47	..	0·033	..	12°·5	Winter frog	Vernon.

An examination of the above results shows that the respiratory exchange of most of the cold-blooded animals is very small, but that a marked exception exists in the case of insects, which have a metabolism equal to that of the larger mammals. This remarkable exception finds confirmation in the relatively high temperature[4] of insects and in their wonderful muscular activity.

[1] "Chem. Untersuch. u. d. Respir. d. Schlammpeitzgers," Breslau, 1855, S. 24.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1873, tome lxxvi. p. 1141.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 78.
[4] Article "Animal Heat," this Text-book, vol. i.

The respiration of fishes.—The necessity of air for the life of fishes was proved by Boyle [1] during his experiments with the air-pump. He states that "there is wont to lurk in water many little parcels of interspersed air, whereof it seems not impossible that fishes may make some use, either by separating it when they strain the matter through their gills, or by some other way." Mayow [2] (1674) appears to have been the first to understand and to correctly describe how fish breathed by taking up nitro-aerial gas (oxygen) from the water by means of the blood flowing through their gills; and Bernouilli [3] in 1690 demonstrated that fish could not live in cold water from which the air had been expelled by boiling.

The methods employed and quantitative results obtained by different observers, who have studied the respiration of fishes and other animals living in water, have already been described. A few additional facts, however, must be mentioned. Humboldt and Provençal [4] state that nitrogen was in some cases absorbed, but when the water was impregnated with oxygen and hydrogen none of the latter gas was taken up by the fishes; a certain amount of cutaneous respiration also occurs, and fishes can breathe in the air as long as their gills are kept moist with water. The respiration of fishes living in the sea is facilitated by the absence of any free carbon dioxide in the water, for any carbon dioxide formed is at once fixed by the excess of alkaline base present in the water.[5]

In connection with the respiration of fishes, the *swimming-bladder* must be considered, for this organ is one which can secrete gases and in some cases store up almost pure oxygen.

Biot [6] found that the percentage of oxygen increased with the depth from which the fish was taken; the greatest percentage was 87. This was confirmed by the observations of Delaroche,[7] who obtained 70 per cent. oxygen from the bladder of fishes drawn up from a greater depth than 50 metres (164 feet), and 29 per cent. from those taken at smaller depths. Erman,[8] Vauquelin,[9] Configliachi,[10] and Delaroche [11] analysed the gas in the swimming-bladder of fresh-water fish, and found the percentage of oxygen generally less than that in the atmosphere, little or no carbon dioxide, but much nitrogen. The mean of analyses made by Humboldt and Provençal showed 7·1 parts of oxygen, 5·2 of carbon dioxide, and 87·7 parts of nitrogen in 100 volumes of gas from the swimming-bladder of a carp, while the results obtained by F. Schultz [12] varied between 1·1 and 13·2 per cent. oxygen and 1·4 and 5·4 per cent. carbon dioxide.

According to Humboldt and Provençal, the tench (*Cyprinus tinca*), in which there is a duct communicating between the air-bladder and the mouth, does not take hydrogen into its bladder when the water in which it is confined is saturated with that gas. Moreau obtained similar negative results; but more

[1] "New Experiments, Physico-Mechanical, touching the Spring of the Air," 1662; *Phil. Trans.*, London, 1670, pp. 2011, 2035; "Works," Shaw's edition, vol. i. p. 109.

[2] "Tractatus quinque," Oxon., 1674, vol. i. ch. xv. p. 259.

[3] "Dissertatio de effervescentia et fermentatione nova hypothesi fundata," ch. xiv. Basiliæ, 1690.

[4] *Mém. de la Soc. de phys. et de chim. d'Arcueil*, Paris, 1809, tome ii. p. 359; *Journ. f. Chem. u. Phys.*, Nürnberg, 1811, Bd. i. S. 86.

[5] Dittmar, *Proc. Phil. Soc. Glasgow*, vol. xvi. p. 61; M'Kendrick, *Nature*, London, 1888, Aug. 16; *Brit. Med. Journ.*, London, 1888, vol. ii. p. 331; Petersen, *Scottish Geograph. Mag.*, Edinburgh, 1895, June, p. 294.

[6] *Mém. de la Soc. de phys. et de chim. d'Arcueil*, Paris, 1807, tome i. p. 252; *Ann. d. Phys. u. Chem.*, Leipzig, Bd. xxvi. S. 454.

[7] *Journ. f. Chem. u. Phys.*, Nürnberg, 1811, Bd. i. S. 122.

[8] *Ann. d. Phys. u. Chem.*, Leipzig, 1808, Bd. xxx. S. 113.

[9] Vauquelin, quoted from Erman, reference 8.

[10] *Journ. f. Chem. u. Phys.*, Nürnberg, 1811, Bd. i. S. 137.

[11] *Ibid.*, S. 164.

[12] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 48.

recent experiments by Mengarini [1] show that the goldfish (*Carassius auratus*) and roach (*Leuciscus*), in which there is a ductus pneumaticus, the mullet (*Mugil cephalus*) and rockling (*Motella*), in which there is no duct, do take up hydrogen from water saturated with that gas.

Moreau [2] has shown that the withdrawal of the gas of the swimming-bladder by means of a trocar leads in a short time to the secretion of a gas richer in oxygen, and that by a repetition of this process the percentage of oxygen can be raised as high as 85 ; he also states that section of the sympathetic nerve hastens the process of secretion. These observations have been repeated and extended by Hüfner [3] and Bohr.[4] Cod-fish (*Gadus callarias*) were caught in a net at a depth of about 14 metres (46 feet), and when they were drawn to the surface the gas in the air-bladder expanded so much that the fish swam with their backs downwards. The gas was found in one case to contain 52 per cent. of oxygen ; but when the fish had been near the surface of the water for some time only 13 per cent. of oxygen was obtained. After the removal of the gas from the bladder, its secretion begins again ; within six hours a little gas has accumulated, and in twenty-four hours the bladder is again full. The rapidity of the process and the increase in the percentage of oxygen is shown by the following examples :—

Number of Experiment.	Hours after the First Puncture.	Percentage of Oxygen.	Percentage of Carbon Dioxide.	Remarks.
24	0	15·0	...	8 c.c. of gas in the air-bladder.
	48	78·5	1·0	$7\frac{1}{3}$ c.c. ,, ,,
	71	83·7	0·5	7 c.c. ,, ,,
25	0	15·0	...	6 c.c. ,, ,,
	50	78·4	0·8	5 c.c. ,, ,,
	73	72·7	1·0	6 c.c. ,, ,,
15	0	16·3	0·1	...
	5	16·3	0·2	...

Bohr has also shown that after section of the branches (rami intestinales) of the vagus which supply the air-bladder, the secretion of the gas ceases entirely, but that no effect upon the secretion is observed after section of the rami cardiaci or the nervi laterales. These phenomena observed in the case of the swimming-bladder can at present be explained only as a process of secretion. The air is not swallowed, for the fishes with the greatest percentage of oxygen in their bladders are those which do not come to the surface, but live at great depths ; in some of them, moreover, such as the cod (*Gadus callarias*), there is no communication between the bladder and the mouth. The phenomena cannot be accounted for by simple diffusion, for the water which surrounds the fish cannot have a higher tension of oxygen than 21 per cent. of an atmosphere.[5] Further, Bohr has shown that the percentage of oxygen is not reduced by diffusion outwards, for when the secretion of fresh oxygen is prevented by section of the vagus, the high percentage of oxygen is maintained for two or three days. As long as the swimming-bladder is fresh, it is almost impermeable to oxygen, even when the difference of pressures inside and outside the bladder amounts to one atmosphere. It is to be noted that the membrane lining the swimming-bladder has a peculiar glandular structure.

[1] *Arch. f. Physiol.*, Leipzig, 1889, S. 54.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1873, tome lvii. pp. 37, 816 ; "Recherches experimentales sur les functiones de la vessie natatoire," Paris, 1876 ; "Mém. de physiol.," Paris, 1877, pp. 69–86.

[3] *Arch. f. Physiol.*, Leipzig, 1892, S. 54.

[4] *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 494 ; *Compt. rend. Acad. d. sc.*, Paris, 1892, tome cxiv. p. 1560.

[5] Biot, Delaroche, Moreau, *loc. cit.* ; Jakobsen, *Ann. d. Chem. u. Pharm.*, Bd. clxvii. S. 1 ; Hüfner, *Arch. f. Physiol.*, Leipzig, 1897, S. 112.

Table of the Respiratory Exchange of Warm-Blooded Animals.

Animal.	Weight (in Grms.).	Oxygen per Kilo. and Hour (in Grms.).	Carbon Dioxide per Kilo. and Hour (in Grms.).	$\frac{CO_2}{O_2}$	Temperature.	Remarks.	Observer.
BIRDS—							
Common Hen	1280	1·058 (740 c.c.)	1·327 (675 c.c.)	·91	19°	Fed on oats and water.	Regnault and Reiset.[1]
,,	,,	1·057 (739 c.c.)	1·403 (714 c.c.)	·96	23°	,,	,,
,,	1578	1·084 (758 c.c.)	1·486 (756 c.c.)	·99	14°	,,	,,
,,	1546	1·067 (746 c.c.)	1·447 (736 c.c.)	·93	19°	,,	,,
,,	1637	1·109 (775 c.c.)	1·561 (793·6)	1·02	19°	,,	,,
,,	1820	..	1·665	·83	..	Mean of 12 experiments on 2 hens.	Richet.[2]
,,	1500	..	1·755	·83	..	Mean of 7 experiments.	,,
Duck . .	1740	..	2·270	·74	..	Mean of 5 experiments.	,,
Goose (4) . .	18,400	·677 (473 c.c.)	·649 (330 c.c.)	·69	16°	Determination lasted 25 hrs., no food given.	Reiset.[3]
,, (1) . .	2975	..	1·490	·80	..	Mean of 12 experiments.	Richet.
Turkey-cock (2) .	12,250	·702 (490 c.c.)	·791 (402 c.c.)	·77	16°	Determination lasted 18½ hrs., no food given.	Reiset.
,, (1) .	2650	..	1·319	·71	..	Mean of 5 experiments.	Richet.
Pigeon . . .	325	..	3·360	·79	..	Mean of 11 experiments.	,,
,, . . .	232–380	..	3·236	..	..	Mean of 10 experiments.	Corin and Van Beneden.[4]
Turtle-dove .	167	..	4·591	..	..	Mean of 11 experiments on 3 doves.	Boussingault.[5]
Greenfinch .	25	13·000 (9091 c.c.)	13·590 (6909 c.c.)	·76	17°	..	Regnault and Reiset.
	25	9·742 (6813 c.c.)	9·246 (4701 c.c.)	·69	17°	..	,, ,,
Goldfinch . .	21·5	..	12·582	·71	..	Mean of 3 experiments.	Richet.
Sparrow . .	22	9·595 (6710 c.c.)	10·492 (5334·5 c.c.)	·79	18°	..	,,
,, . .	25	..	7·783 (3957 c.c.)	..	10°–15°	Mean of two determinations.	Pott.[6]
Crossbill . .	28·6	10·974 (7674 c.c.)	12·014 (6108·5 c.c.)	·79	17°	..	Regnault and Reiset.
MAMMALIA—							
Rabbit . .	2755	0·987 (690 c.c.)	1·244 (632 c.c.)	·91	21°–22°	..	,,
,, . .	2780	0·877 (613 c.c.)	1·107 (563 c.c.)	·92	23°	..	,,
,, . . .	4140	0·797 (557 c.c.)	1·039 (528 c.c.)	·95	,,	..	,,
,, . . .	1433	1·012	1·354	·97	18°–20°	Fed on swedes.	Pembrey and Gürber.[7]
,, . . .	1882	0·762	0·943	·90	,,	,,	,,
,, . . .	1931	0·883	1·142	·94	,,	,,	,,
Guinea-pig . .	..	..	1·35	..	..	Mean of 6 experiments.	Marchand.[8]

[1] *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi.
[2] *Arch. de physiol. norm. et path.*, Paris, 1890, tome xxii. p. 485.
[3] *Ann. de chim. et phys.*, Paris, 1863, Sér. 3, tome lxix. p. 129.
[4] *Trav. du lab. de Liège*, 1888, tome i. p. 110.
[5] *Ann. de chim. et phys.*, Paris, 1844, Sér. 3, tome xi. p. 444.
[6] *Landwirthsch. Versuchsstat.*, Bd. xviii. S. 81.
[7] *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 449.
[8] *Journ. f. prakt. Chem.*, Leipzig, 1848, Bd. xliv. S. 1.

TABLE—*continued.*

Animal.	Weight (in Grms.).	Oxygen per Kilo and Hour (in Grms.).	Carbon Dioxide per Kilo. and Hour (in Grms.).	$\frac{CO_2}{O_2}$	Temperature.	Remarks.	Observer.
MAMMALIA—							
Guinea-pig[2] .	..	1·612	1·896	·86	18·8	..	Colasanti.[1]
,, . .	444·9	1·478	1·758	·86	22°	Duration of experiment was 2¼ hours.	Pembrey.
,, . .	445·6	1·416	1·885	·96	20°	,,	,,
Dog . . .	6213	1·303 (911 c.c.)	1·325 (674 c.c.)	·74	21°	Fed on raw meat.	Regnault and Reiset.
,, . . .	6158	1·393 (975 c.c.)	1·425 (724 c.c.)	·74	15°	,,	,,
,, . . .	20,000–28,000	..	1·026	·748	..	Mean of experiments on 4 dogs.	Richet.[3]
,, . . .	13,000–14,000	..	1·210	·748	..	Mean of experiments on 5 dogs.	,,
,, . . .	11,000–12,000	..	1·380	·748	.	Mean of experiments on 7 dogs.	,,
,, . . .	8000–10,000	..	1·506	·748	..	Mean of experiments on 4 dogs.	,,
,, . . .	6000–7000	..	1·624	·748	..	Mean of experiments on 3 dogs.	,,
,, . . .	4700–5600	..	1·688	·748	..	,,	,,
,, . . .	2800–3800	..	1·964	·748	..	Mean of experiments on 6 dogs.	,,
,, . . .	2200–2500	..	2·650	·748	..	Mean of experiments on 4 dogs.	,,
,, . . .	34,000	..	0·709	..	..	..	Leyden and Fränkel.[4]
,, . . .	33,000	..	0·668	..	..	Mean of 17 experiments.	Pettenkofer and Voit.[5]
,, . . .	18,000	..	1·230	..	..	..	Gréhant and Quinquand.[6]
,, . . .	6750	..	0·939	..	..	..	Wood.[7]
,, . . .	5300	..	0·690	..	..	Mean of 9 experiments.	Senator.[8]
,, . . .	5200	..	1·288	..	..	Mean of 7 experiments.	Bauer and Bœck.[9]
,, . . .	4000	..	1·126	..	..	Mean of 4 experiments.	Page.[10]
Cat . . .	2464–3047	1·356 (947 c.c.)	1·397 (710 c.c.)	..	–3°·2	..	Carl Theodor.[11]
,, . . .	,,	0·645 (450 c.c.)	0·766 (389 c.c.)	..	29°·6	..	,,
,, . . .	..	..	1·364 (693 c.c.)	..	..	Liberal diet of meat.	Bidder and Schmidt.[12]
,, . . .	..	..	1·423 (723 c.c.)	..	..	,,	,,
Sheep . . .	66,000	0·490 (343 c.c.)	0·671 (341 c.c.)	·99	16°	Exprmnt. lasted 14⅓ hours; no food during that time.	Reiset.
,, . . .	..	..	0·733	..	..	..	Henneberg.[13]
Ox . . .	638,000–660,000	..	0·389–0·485	..	..	..	,,
,, . . .	710,000	..	0·488–0·616	..	..	..	,,

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. iv. S. 92 and 469.

[2] See also Finkler, *ibid.*, Bonn, 1877, Bd. xv. S. 603.

[3] *Compt. rend. Acad. d. sc.*, Paris, 1889, tome cix. p. 190; *Arch. de physiol. norm. et path.*, Paris, 1890, tome xxii. p. 17.

[4] *Virchow's Archiv*, 1879, Bd. lxxvii. S. 136.

[5] *Ztschr. f. Biol.*, München, 1873, Bd. ix. S. 1.

[6] *Journ. de l'anat. et physiol., etc.*, Paris, 1882, tome xviii. p. 469.

[7] Fever, *Smithson. Contrib. Knowl.*, Washington, 1880.

[8] *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1872, S. 1.

[9] *Ztschr. f. Biol.*, München, 1874, Bd. x. S. 341.

[10] *Journ. Physiol.*, Cambridge and London, 1879, vol. ii. p. 228.

[11] *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 51.

[12] "Die Verdauungssäfte und der Stoffwechsel," Leipzig, 1852, S. 321–362.

[13] *Landwirthsch. Versuchsstat.*, 1866, Bd. viii. S. 443; "Neue Beitr. z. Begründung einer rationellen Fütterung der Wiederkäuer," Göttingen, 1870–72.

TABLE—*continued.*

Animal.	Weight (in Grms.).	Oxygen per Kilo. and Hour (in Grms.).	Carbon Dioxide per Kilo. and Hour (in Grms.).	$\frac{CO_2}{O_2}$	Temperature.	Remarks.	Observer.
MAMMALIA—							
Boar . . .	135,000	0·391 (273 c.c.)	0·443 (225 c.c.)	·82	16°	Exprmnt. lasted 13½ hrs.; food during the experiment.	Reiset.
Sow . . .	105,000	0·561 (392 c.c.)	0·661 (336 c.c.)	·85	17°·9	,,	,,
Rat (white) . .	80·5	..	3·518 (1789 c.c.)	..	7°	..	Pott.
,, (grey) . .	55·5	..	4·308 (2190 c.c.)	..	16°	..	,,
Mouse (white) .	13	..	8·880 (4514 c.c.)	..	7°	..	,,
,, ,, .	25	..	8·4	..	17°	..	Pembrey.[1]
,, (common)	19·2	6·660	7·443	·80	10°·5	..	Oddi.[2]
Man . . .	70,000–73,000	..	0·41	..	..	Minimum and maximum in 24 hrs.; man at rest.	Pettenkofer and Voit.[3]
,, . . .	,,	..	0·61	..	..		,,
,, . . .	,,	..	0·76	..	..	Man at work.	,,
,, . . .	71,000–74,220	..	0·373	..	..	Hunger.	Ranke.[4]
,, . . .	,,	..	0·52	..	..	Very liberal diet.	,,
,, . . .	65,500	..	0·512	..	..	..	Scharling.[5]
,, . . .	82,000	..	0·497	..	..	..	,,
,, . . .	57,750	..	0·594	..	..	..	,,
,, . . .	57,000–60,000	0·601 (420 c.c.)	0·717 (364 c.c.)	·98	..	Max.	Speck.[6]
,, . . .	,,	0·461 (322 c.c.)	0·535 (271 c.c.)	·82	..	Min. Of 12 experiments.	,,
,, . . .	,,	0·516 (361 c.c.)	0·619 (314 c.c.)	·87	..	Mean	,,
,, . . .	50,000	17,000 c.c.	13,000 c.c.	·77	..		
,, . . .	,,	16,000 ,,	13,300 ,,	·83	..	Per hour and 50 kilos.	Hanriot and Richet.[7]
,, . . .	,,	18,200 ,,	13,550 ,,	·75	..		
,, . . .	67,500	222·9 ,,	202·7 ,,	·88	..	Hunger(per minute and 67½ kilos.).	Löwy.[8]
,, . . .	60,500	247·2 ,,	196·1 ,,	·79	..	Hunger(per minute and 60½ kilos.).	,,
,, . . .	59,000	3·53 ,,	2·88 ,,	..	14°–19°·8		Geppert.[9]
,, . . .	96,000	3·27 ,,	2·28 ,,	..	16°·4–19°·9	Man at rest (per kilo. and minute).	,,
,, . . .	72,000	4·41 ,,	3·47 ,,	..	17°·8–19°·2		,,

[1] *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 401.
[2] *Arch. ital. de biol.*, Turin, 1891, tome xv. p. 223.
[3] *Ann. d. Chem. u. Pharm.*, 1867, Bd. cxli. S. 295; *Ztschr. f. Biol.*, München, 1866, Bd. ii. S. 459; 1869, Bd. v. S. 319; *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Nov. 10, 1866; Feb. 9, 1867.
[4] *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1862, S. 311.
[5] *Ann. d. Chem. u. Pharm.*, 1843, Bd. xlv. S. 214.
[6] "Untersuch. ueber Sauerstoffverbrauch. u. Kohlensäureausathmung des Menschen," Cassel, 1871, S. 31.
[7] *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvi. p. 419.
[8] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. S. 523, 524.
[9] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 381.

The respiratory exchange of warm-blooded animals.—The tissues of warm-blooded animals are the seat of a very energetic combustion, which is subject to quantitative and qualitative changes, owing to the influence of certain factors, such as age, size of body, external temperature, muscular activity, rest, digestion, hunger, and hibernation. A general comparison between the various members of the two great classes of the warm-blooded animals, birds and mammals, will be found in the tables on pp. 706–708.

These tables[1] show that, weight for weight, birds have a more rapid respiratory exchange than mammals, and this difference is associated with a higher bodily temperature.[2] It is also to be noticed that the respiratory quotient of the herbivorous animals is nearly unity, but that of the carnivorous animals is about 0·74. The respiratory exchange of small animals of the same or of different species is relatively greater than that of large animals.[3] The causes of many of these differences will now be discussed in detail.

The influence of external temperature upon the respiratory exchange.—Since the time when Crawford[4] showed by experiment that external cold increased the discharge of carbon dioxide from a warm-blooded animal, numerous similar observations have been made by various observers. The most important result of this work has been the discovery that cold-blooded animals respond to changes of external temperature in an exactly opposite way to that shown by warm-blooded animals; in the former class a rise or fall in the temperature of the surroundings produces respectively an increase or decrease in the intake of oxygen and the output of carbon dioxide, whereas in the latter class cold increases and heat diminishes the respiratory exchange. On this account it will be well to consider separately the influence of temperature on these two classes of animals, and then to discuss the causes of the great difference in the effect.

Cold-blooded animals.—Some of the earliest experiments upon the influence of temperature upon the respiratory exchange of cold-blooded animals appear to have been made by Delaroche,[5] Treviranus,[6] and Marchand,[7] but, owing to imperfect methods, their results are not very exact, although they show that the respiratory exchange slowly rises and falls with the external temperature.

In 1857, Moleschott[8] made a series of experiments upon frogs, and found that exposure to an increased external temperature or to light caused an increase in the output of carbon dioxide.

Regnault and Reiset[9] made three observations upon the respiratory exchange of green lizards at different external temperatures, and obtained the following results:—

[1] Further data will be found in the article by Zuntz, Hermann's "Handbuch," Bd. iv. Th. 2, S. 129, from which many of the figures in the above tables have been taken. See also tables in paper by Richet, *Arch. de physiol. norm. et path.*, Paris, 1891, tome xxiii. p. 74.

[2] Article "Animal Heat," this Text-book, vol. i. p. 791.

[3] See p. 720.

[4] "On Animal Heat," London, 1788, pp. 311, 387.

[5] *Journ. de phys. de chim., etc.*, Paris, 1813, tome lxxvii. p. 5.

[6] *Ztschr. f. Physiol.*, 1831, Bd. iv. S. 1.

[7] *Journ. f. prakt. Chem.*, Leipzig, Bd. xxxiii. S. 152.

[8] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1857, Bd. ii. S. 315.

[9] *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi.

Weight.	Oxygen per Kilo. and Hour.		Carbon Dioxide per Kilo. and Hour.		$\frac{CO_2}{O_2}$	Nitrogen discharged per Kilo. and Hour.	Temperature.	Remarks.
Grms.	Grms.	C.c.	Grms.	C.c.		C.c.		
3 lizards, 68·5	0·0246	17·2	0·025	12·6	·73	5·732	7°·3	Hibernating.
2 ,, 42	0·0646	45·2	0·063	32·3	·71	1·905	14°·8	Half awake.
3 ,, 62	0·1916	134·0	0·199	100·77	·75	2·49	23°·4	Awake and well; fed for a month.

There are, however, several conditions which prevent these results from being considered comparable; the hibernating[1] animal has a very low respiratory exchange, even when the external temperature is higher than 7°·3; in the last experiment the food would increase the respiratory exchange; the observations were made at intervals of several months, and are complicated by the large discharge of nitrogen, which is probably to be attributed to an error of experiment.[2]

Bütschli[3] showed that the respiratory exchange of insects varied in the same direction as the temperature of their surroundings.

The most complete series of observations appear to be those of Schulz[4] upon the edible frog (*Rana esculenta*). The following table gives his chief results, obtained upon frogs in summer:—

Temperature of the Respiration Chamber.	Temperature of Frog.	CO_2-Output per Kilo. and Hour. C.c. at 0° and 760 Mm.	CO_2-output per Kilo. and Hour in Grms.
0°·0	1°·0	4·31	0·0084
0°·25	1°·0?	6·097	0·0119
0°·8	1°·5	7·50	0·0147
6°·1	6°·4	34·17	0·0672
15°·8	15°·4	35·30	0·0694
17°·0	15°·2	41·83	0·0822
25°·5	25°·0	76·26	0·1499
25°·5	25°·3	86·75	0·1706
33°·0	33°·0	279·40	0·5495
33°·2	33°·1	314·53	0·6179
34°·2	33°·5	340·48	0·6696
35°·0	34°·0	325·05	0·6392

It is to be noted that in Schulz's experiments the frogs were kept in the warm or cold surroundings until their temperature was equal to that of the air, so that the results are strictly upon frogs at different temperatures. The response of a frog, as shown by its temperature and respiratory exchange, to a change of external temperature is very slow, and for this reason observations upon the metabolism of cold-blooded animals can only be properly compared when the temperature of the animal and that of the air are known. The above results show that at temperatures a degree or two above zero the output of carbon dioxide

[1] See "Animal Heat," this Text-book, vol. i.
[2] See also Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 73.
[3] *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1874, S. 348.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 78.

is very minute, and rises with the temperature, until at 34° the output is, weight for weight of body, equal to that of a man.

The observations of Pembrey[1] and Vernon[2] seem to show that the output of carbon dioxide in frogs (*Rana temporia*) does not increase in exact proportion with the temperature.

Warm-blooded animals.—Since the first experiments by Crawford,[3] in which it was shown that a guinea-pig produced more carbon dioxide in cold than in warm surroundings, numerous observations[4] have been

Animal.	Temperature of Air.	Oxygen absorbed per Kilo. and Hour.	Carbon-Dioxide discharged per Kilo. and Hour.	Water discharged per Kilo. and Hour.	$\frac{CO_2}{O_2}$	$\frac{CO_2}{H_2O}$	Observer.
		C.c.	C.c.				
Guinea-pig	15°·5	1079·66	1065·92	...	·98	...	Colasanti.[5]
	6°·0	1438·31	1262·67	...	·88	...	
,,	22°·2	1050·00	867·19	...	·83	...	
	6°·3	1592·33	1230·00	...	·79	...	
,,	3°·64	1856·50	1554·80	...	·83	...	Finkler.[6]
	26°·21	1118·50	1057·40	...	·94	...	
		Grms.	Grms.	Grms.			
Mouse; weight about 19 grms.	3°	9·030	9·505	6·705	·76	1·4	Oddi.[7]
	5°	8·384	8·641	6·721	·74	1·2	
	10°·5	6·660	7·443	5·079	·80	1·4	
	25°	4·862	5·400	5·102	·80	1·0	
	35°	5·912	4·977	4·736	·65	1·0	
Pigeon	−9°	...	3·016	...	...	...	Corin and Van Beneden.[8]
	17°·5	...	1·141	...	...	...	
		Grms.	Grms.	Grms.			
Cat	−3°·2	21·39	22·03	14·12	...	...	Carl Theodor.[9]
	1°·3	17·73	18·92	10·87	...	...	
	18°·0	12·30	13·93	10·60	...	...	
	29°·6	13·91	13·12	19·48	...	...	
		For periods of six hours, cat weighing 2557 - 2650 grms. The figures are *not* reduced to kilo. and hour.					

[1] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi.

[2] *Journ. Physiol.*, Cambridge and London, 1894–1895, vol. xvii. p. 277.

[3] "On Animal Heat," London, 1788, pp. 311, 387.

[4] Delaroche, *Journ. de phys. de chim. etc.*, Paris, 1813, tome lxxvii. p. 5; Vierordt, "Physiol. des Athmens," 1845; Wagner's "Handwörterbuch," 1844, Bd. ii. S. 828; Letellier, *Ann. de chim. et phys.*, Paris, 1845, Sér. 3, tome xiii. p. 478; Lehmann, *Abhandl. d. k. sächs. Gesellsch. d. Wissensch.*, Leipzig, 1846, S. 463; Liebermeister, *Deutsches Arch. f. klin. Med.*, Leipzig, 1872, Bd. x. S. 89, 420; Gildemeister, *Virchow's Archiv*, Bd. lii. S. 130; Sanders-Ezn, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Cl.*, Leipzig, 1867, S. 58; Röhrig and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 57; Pflüger, *ibid.*, 1876, Bd. xii. S. 282; Regnault and Reiset, *Ann. d. Chem. u. Pharm.*, 1850, Bd. lxxiii. S. 260; Berthollet, *Mém. de la Soc. de phys. et de chim. d'Arcueil*, Paris, tome ii.; Senator, *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1872, S. 1; 1874, S. 42, 54; *Centralbl. f. d. med. Wissensch.*, Berlin, 1871, Nos. 47 and 48; Erler, *Arch. f. Anat., physiol., u. wissensch. Med.*, 1876, S. 556; Litten, *Virchow's Archiv*, 1877, Bd. lxx. S. 10; Fredericq, *Arch. de biol.*, Gand, 1882, tome iii. pp. 736, 743; Quinquand, *Compt. rend. Acad. d. sc.*, Paris, 1887, tome civ. p. 1542.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 92. See also Pflüger, *ibid.*, S. 469.

[6] *Ibid.*, 1877, Bd. xv. S. 603.

[7] *Arch. ital. de biol.*, Turin, 1891, vol. xv. p. 223.

[8] *Arch. de biol.*, Gand, 1887, tome vii. p. 265.

[9] *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 51.

made upon the influence of external temperature upon the respiratory exchange of warm-blooded animals. The general result of this work is that the intake of oxygen and the output of carbon dioxide increase with a fall and decrease with a rise of external temperature. This is shown by the examples, which have been taken from the results obtained by different observers, and are given in the preceding table.

It appears that, when the external temperature is raised to a point about 30°, the respiratory exchange shows an increase above the amount observed at a temperature of 20°. Thus Voit[1] found in the case of a man, that the output of carbon dioxide was increased by a fall of 9° or 10° below the average temperature 14°–15°, and also increased by a rise of 15° or 16° above that point; the augmentation in the discharge of carbon dioxide was respectively 36 per cent. and 10 per cent. above that given off at 14°–15°. A similar result was obtained by Page,[2] who found that at a temperature of 25° the discharge of carbon dioxide by a dog was at a minimum; a fall or rise of 10° below that point produced a mean increase of 31 per cent. and 51 per cent. respectively.[3] Unfortunately Voit gives no details as to the temperature of the man during the experiments, but in one or two cases Page notes that the temperature of the dog was raised above the normal by exposure to the warm air.

The earliest experiments upon the influence of external temperature on the respiratory exchange of man were made by Lavoisier and Seguin,[4] who found that a man at rest absorbed in an hour 34·49 grms. of oxygen when the air was 32°·5, but 38·31 grms. when the temperature was 15°. Since that time many observations[5] have been made upon man and the effect of external temperature on his respiratory exchange, and of these the most important are those made by Löwy.[6] The general result drawn from his experiments is that the effect of external cold varies in different men. Out of fifty-five experiments, the oxygen absorbed was increased above 5 per cent. in twenty-six cases, unaltered in twenty, and diminished in nine cases. In these experiments, in which the metabolism was increased, for the variations in the output of carbon dioxide followed those in the absorption of oxygen, the heights to which it was raised varied between 5 and 90·8 per cent. above the normal. A point worthy of note is that the greatest increase in the respiratory exchange was observed in the men who shivered or moved when they felt cold, and that the respiratory exchange remained unaltered or decreased in the men who, notwithstanding the sensation of cold, remained quiet, and by an effort of the will suppressed any tendency to move or shiver. Löwy concludes that the only involuntary regulator of temperature in a man exposed to moderate cold is the skin. It must be pointed out, however, that increased muscular activity in a man who

[1] *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 80.

[2] *Journ. Physiol.*, Cambridge and London, 1879–80, vol. ii. p. 228.

[3] See also Rubner, "Biologische Gesetze," Universitäts-programm, Marburg, 1887; abstract in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, S. 700.

[4] "Œuvres de Lavoisier," tome ii. pp. 688, 704; *Hist. Acad. roy. d. sc.*, Paris, 1789, p. 575. See also *Rep. Brit. Ass. Adv. Sc.*, London, 1871, p. 189.

[5] Vierordt, "Physiol. des Athmens," 1845; E. Smith, *Phil. Trans.*, London, 1859, vol. cxlix., p. 681; Speck, *Schrift. d. Gesellsch. z. Beförd. d. ges. Naturw. zu Marburg*, 1871, Bd. x.; Liebermeister, *Deutsches Arch. f. klin. Med.*, Leipzig, 1872, Bd. x. S. 89, 420; Lehmann, *Virchow's Archiv*, 1873, Bd. lviii. S. 92. Johansson, *Skandin. Arch. f. Physiol.*, Leipzig, 1897, Bd. vii. S. 123.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 189.

feels cold, is not necessarily brought about by a conscious effort of the will; it is to a great extent reflex, and shows itself in the more energetic performance of work, or, if no work be done, the reflex may become so imperative as to give rise to involuntary movement, shivering, which is only of value to the organism as a source of greatly increased heat production. There is little doubt but that a normal man, who feels cold and is free to follow the dictates of his sensations, will be more active, and will produce more carbon dioxide and absorb more oxygen than he would in warm surroundings. The man who suppresses increased muscular action when he feels cold, is abnormal. It follows, therefore, that man is no exception to the general rule that warm-blooded animals in cold surroundings increase, in warm surroundings diminish, their respiratory exchange and production of heat.

It has already been shown that a rise or fall in external temperature determines in the same direction a variation of the respiratory exchange of cold-blooded animals. What, then, is the cause of the totally opposite result observed in warm-blooded animals? To this question only an incomplete answer can be given. The difference is due to the nervous and muscular mechanisms which maintain the fairly constant temperature observed in the warm-blooded animals. For if, as Sanders-Ezn[1] and Pflüger[2] have shown, the exposure to cold be excessive, and the animal's temperature falls to 26°, then also there is a fall in the intake of oxygen and the output of carbon dioxide; on the other hand, if by means of warm baths the internal temperature of the animal is raised above the normal, then there is an increase above the average respiratory exchange. In fact, a warm-blooded animal responds to a rise or fall in the temperature of its surroundings with a decrease or increase of its metabolism, only as long as its internal temperature remains near the normal point. Moreover, Pflüger has proved the connection between the normal response to a change of external temperature and the nervo-muscular system, for he shows that a mammal paralysed with curari[3] or with its spinal cord cut in the lower cervical region, absorbs more oxygen and discharges more carbon dioxide in warm than in cold surroundings; it resembles in this respect a cold-blooded animal. A similar cold-blooded condition can be produced in mammals, as Rumpf,[4] Richet,[5] and Pembrey[6] have observed, by exposing the anæsthetised animal to changes of temperature.

The objection that these experiments are associated with markedly abnormal conditions, and therefore cannot indicate the true condition of normal animals, is met by the fact that it is possible to trace the gradual development of the means whereby an animal increases or decreases its metabolism and maintains a fairly constant heat of its body, notwithstanding wide variations in the temperature of its surroundings. This has been shown by Pembrey[7] in a series of comparative experiments upon full-grown and newly-born animals. In the full-grown mouse the response to a change of external temperature

[1] *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Kl.*, Leipzig, 1867, S. 58.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii. S. 247.
[3] See also Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 522.
[4] *Ibid.*, 1884, Bd. xxxiii. S. 538.
[5] *Compt. rend. Acad. d. sc.*, Paris, 1889, tome cix. p. 190.
[6] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894–95, vol. xvii.
[7] *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 401; 1895, vol. xviii. p. 363.

is almost immediate. The contrast in the case of young mice of different ages is shown by the fact that a fall in external temperature produces a fall in the output of carbon dioxide, and in the temperature of the young mouse, until it is about nine days old, when it begins to respond in a similar way to that observed in a full-grown animal.

A similar development can be observed in other young animals born in an immature condition, and in the chick[1] before and after it is hatched, but a marked contrast is found in young animals born with a well-developed and active body.[2]

The influence of muscular activity upon the respiratory exchange.—Muscular activity greatly increases the rate of breathing, the intake of oxygen, and the output of carbon dioxide. It was but natural, therefore, that physiologists should attribute the hyperpnœa caused by excessive muscular exertion to a deficiency of oxygen, or to an accumulation of carbon dioxide in the blood, consequent upon the greatly increased metabolism. This theory, however, has been proved by experiment to be erroneous. Mathieu and Urbain[3] determined the gases present in samples of blood removed from an animal after a period of rest, and again after a period of activity, and they found as a general result an increase in the oxygen, and a decrease in the carbon dioxide of the blood in the latter condition. Their analyses, however, were subject to certain sources of error. The question has been more thoroughly investigated by Geppert and Zuntz,[4] who found that muscular activity is indeed accompanied by an increase in the oxygen and a decrease in the carbon dioxide of the blood, and that the hyperpnœa is probably due to some product of muscular activity which is absorbed by the blood and carried to the medulla oblongata, where it stimulates the respiratory centre. The chief evidence for these statements will now be given. After section of the spinal cord of a dog in the dorsal region, tetanisation of the hind limbs causes an increase in the air inspired, in the intake of oxygen, and in the output of carbon dioxide.[5]

Dog weighing 2100 *Grms.*

Volume of Air inspired per Minute.	Intake of Oxygen.	Output of Carbon Dioxide.	$\frac{CO_2}{O_2}$	Condition.
	Per Kilo. Body Weight and per Minute.			
1012 c.c.	20·4 c.c.	18·2 c.c.	·89	Rest.
2148 ,,	36·8 ,,	31·8 ,,	·86	Tetanus.
863 ,,	21·6 ,,	16·2 ,,	·75	Rest.
1326 ,,	29·5 ,,	19·3 ,,	·66	Tetanus.

[1] Pembrey, Gordon, and Warren, *Journ. Physiol.*, Cambridge and London, 1894–95, vol. xvii. p. 331.

[2] See also "Animal Heat," this Text-book, vol. i. p. 803.

[3] *Arch. de physiol. norm. et path.*, Paris, 1871–72, tome iv.; *Compt. rend. Acad. d. sc.*, Paris, 1872, tome lxxiv. p. 190.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 189.

[5] See also Hanriot and Richet, *Compt. rend. Acad. d. sc.*, Paris, 1888, p. 75.

Analyses of the gases of blood taken from an animal after voluntary or involuntary muscular exertion show an increase in the oxygen and a decrease in the carbon dioxide.

Gases of the Arterial Blood.

Oxygen Volumes Per Cent.		Carbon Dioxide Volumes Per Cent.		Animal.
Rest.	Activity.	Rest.	Activity.	
17·58	...	38·57	...	Dog.
17·33	17·68	36·49	35·01	
15·88	More than 16·04	53·71	39·06	Rabbit.

Further, if the aorta be compressed in order to shut out the blood from the stimulated limbs, no hyperpnœa is caused by the muscular activity; section of the vagi, sympathetic and recurrent nerves, or section of the cord high up, does not prevent the stimulating effect of muscular exertion upon the respiratory centre. In rabbits the alkalinity of the blood is diminished by the acid formed during tetanic muscular activity, and this is probably a cause of the decrease in the carbon dioxide of the blood. No alteration could be found in the tension of the oxygen and carbon dioxide present in the blood removed from an animal after muscular exertion.[1]

Lehmann[2] has shown that the injection of normal solution of tartaric acid stimulates and quickens the respiration of a rabbit, whereas a normal solution of sodium hydrate depresses the respiratory centre. According to Löwy's[3] experiments, the unknown substance which stimulates the respiratory centre during muscular activity is not excreted by the kidneys, and is not carbon dioxide; for whereas the rate of respiration is doubled by muscular work when the increase above the normal amount of carbon dioxide in the expired air is only 0·5 per cent., yet the same amount of dyspnœa can be produced during rest only by artificially raising the percentage of carbon dioxide to a much higher point, about 5 per cent.

The credit of the discovery that work is associated with an increase in the respiratory exchange, is due to Lavoisier,[4] who, in a series of experiments with Seguin, found that a man at work absorbed 91·2 grms. of oxygen in an hour, whereas at rest he only absorbed 38·3 grms. Although Vierordt[5] and Scharling[6] both observed a similar increase in the output of carbon dioxide in men at work, the first series of careful experiments on the subject were those performed by

[1] For criticism see Speck, *Deutsches Arch. f. klin. Med.*, Leipzig, 1891, Bd. xlvii. S. 509; for reply by Zuntz and Geppert, *ibid.*, 1891, Bd. xlviii. S. 444.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 284.

[3] *Ibid.*, S. 281; 1890, Bd. xlvii. S. 601.

[4] *Hist. Acad. roy. d. sc.*, Paris, 1789, p. 185; "Œuvres de Lavoisier," tome ii. pp. 688-696.

[5] "Physiol. des Athmens," Karlsruhe, 1845; *Arch. f. physiol. Heilk.*, Stuttgart, Bd. iii. S. 536; Wagner's "Handwörterbuch d. Physiol.," 1844, Bd. ii. S. 828.

[6] *Ann. d. Chem. u. Pharm.*, 1843, Bd. xlv. S. 214; *Journ. f. prakt. Chem.*, Leipzig, Bd. xlviii. S. 435.

E. Smith.[1] He found that a man produced 161·6 c.c. of carbon dioxide per minute when he was perfectly at rest, as in a deep sleep; that during a walk at the rate of two miles (3048 metres) an hour, the discharge of carbon dioxide was increased to 569·5 c.c., and to 851·2 c.c. when the rate of walking was quickened to three miles (4571·9 metres) an hour. The greatest increase, 1581·9 c.c. of carbon dioxide per minute, was caused by work upon a treadmill.

In 1866, Pettenkofer and Voit[2] performed a series of important observations upon the metabolism of healthy men, under different conditions as regards work and diet, and they found that if unity represent the value of the output of carbon dioxide and the intake of oxygen when the man is at rest, then work brings about the following results:—

	During Hunger.	Moderate Diet.
Carbon dioxide . .	2·3	1·6
Oxygen	2·1	1·8

The numerous experiments made by Speck,[3] under different conditions as regards the amount and nature of the work performed, show that the air inspired, the oxygen absorbed, and the carbon dioxide discharged, are greatly increased; the percentage composition of the expired air is but little altered, and the respiratory quotient increases slightly during the work. Hanriot and Richet[4] find for each kilogrammetre of work performed by a man, an increase of 3·168 c.c. in the oxygen absorbed, and 4·221 c.c. in the carbon dioxide discharged.

In experiments upon horses, Zuntz and Lehmann[5] obtained the following results:—

	Litres per Minute.			$\frac{CO_2}{O_2}$.
	Air expired.	Carbon Dioxide discharged.	Oxygen absorbed.	
Rest	44	1·478	1·601	·92
Walk	177	4·342	4·766	·90
Trot	333	7·516	8·093	·93

It is impossible here to discuss fully the quantitative relationship between metabolism and work, but the conclusions reached by Katzenstein[6] are as follows:—The work performed by the arms in turning a wheel produces, per unit of work done, a greater increase in the respiratory exchange than walking or climbing; the absorption of oxygen is per unit of work performed somewhat greater for light, than for heavy work; the absorption of oxygen and the discharge of carbon dioxide increase

[1] *Phil. Trans.*, London, 1859, vol. cxlix. pt. 2, p. 681.

[2] *Ztschr. f. Biol.*, München, 1866, Bd. ii. S. 459. See also this article, p. 718, and article by Voit in Hermann's "Handbuch," Bd. vi. Th. 1, S. 201.

[3] *Deutsches Arch. f. klin. Med.*, Leipzig, 1889, Bd. xlv. S. 461.

[4] *Compt. rend. Acad. d. sc.*, Paris, 1887, tome civ. p. 1865; tome cv. p. 76.

[5] *Landw. Jahrb.*, 1889, Bd. xviii. S. 1; *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 396.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlix. S. 380.

equally under ordinary conditions, so that the respiratory quotient remains practically unaltered. It is only immediately after work that the respiratory quotient increases, and becomes sometimes greater than unity. The absorption of oxygen needed for the work of walking on level ground is, per kilo. body weight and minute, 0·1682 c.c. maximum, and 0·0858 c.c. minimum; for each kilogrammetre of work performed in climbing, 1·5036 c.c. maximum, and 1·1871 minimum; and similarly, for turning the wheel, 1·957 c.c.

Löwy [1] shows that there is no definite value which can be assigned to the increase of the respiratory exchange for the performance of a given quantity of work under all circumstances, for the metabolism depends upon the activity of the muscle, which varies under different conditions. Active muscle working under favourable conditions performs its work economically; fatigued muscle working under unfavourable conditions is the seat of an extravagant metabolism.[2]

The decrease observed in the respiratory exchange of animals under the influence of chloroform, ether, chloral, and curari, is to be attributed chiefly to the great decrease in the activity of the muscles.[3]

The influence of food upon the respiratory exchange.—The effect of a meal upon the respiratory exchange is to cause a marked increase in the intake of oxygen and the output of carbon dioxide; this is due

Number of Experiments.	Mean Output of Carbon Dioxide per Kilo. and Hour.		Condition of Animal.
	Grms.	C.c.	
7	1·423	723	Pregnant; very liberal diet of meat.
52	0·902	458	The same animal; hunger for 18 dys.
4	0·998	507	,, ,, 15 ,,
4	0·732	372	,, ,, 18 ,, shortly before death.
6	0·847	430	Male, kept at constant weight with diet of meat.
2	1·364	693	Male, maximal diet of meat.
23	0·888	451	Male, normal diet of meat, but without water.
12	0·679	345	Male, inanition, but with water supplied.
7	1·702	865	Female, not full grown; diet of meat.
6	1·500	763	Female, diet of fat.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlix. S. 405. See also, Gruber, *Ztschr. f. Biol.*, München, 1891, Bd. xxviii. S. 466.

[2] For further experiments upon the influence of work upon the respiratory exchange of (*a*) man, see Hanriot and Richet, *Ann. d. chim. et phys.*, Paris, 1891, Sér. 6, tome xxii. p. 495; and *Trav. du lab. de Ch. Richet*, 1894, tome 1; (*b*) animals, Grandis, *Arch. ital. de biol.*, Turin, 1889, tome xii. p. 237; Smith, *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 65. Criticism of the same by Zuntz and Lehmann, *ibid.*, p. 396; Gréhant, *Compt. rend. Soc. de biol.*, Paris, 1891, p. 14.

[3] Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 522; Pflüger, *ibid.*, 1878, Bd. xviii. S. 247; Rumpf, *ibid.*, 1884, Bd. xxxiii. S. 538; Saint Martin, *Compt. rend. Acad. d. sc.*, Paris, 1887, tome cv. p. 1126; Richet, *ibid.*, 1889, tome cix. p. 190; *Arch. de physiol. norm. et path.*, Paris, 1890, tome ii. p. 221; Pembrey, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894–95, vol. xvii.

not only to the chemical changes which take place in the food during digestion and absorption, but also to the increased glandular and muscular activity of the alimentary canal.[1]

Although Lavoisier[2] knew that food greatly increased the respiratory exchange, the first experiments of importance in this connection are those of Bidder and Schmidt,[3] who made numerous observations upon cats; the results of some of their experiments are given in the preceding table.

In the case of man and other animals, the influence of food of various kinds, and of fasting, has been studied by Pettenkofer and Voit,[4] Senator,[5] Henneberg,[6] Leyden and Fränkel,[7] Fredericq,[8] and others;[9] the general result is that a meal causes an increase in the intake of oxygen and the output of carbon dioxide, whereas a day of fasting causes a decrease. The average results obtained upon man by Pettenkofer and Voit[10] are as follows:—

	Carbon Dioxide.		Oxygen.[11]	
	Day.	Night.	Day.	Night.
	Grms.	Grms.	Grms.	Grms.
1. Fasting—Rest	403	314	435	326
,, Work for nine hours out of twelve	930	257	922	150
2. Moderate Diet—Rest	533	395	443	449
,, ,, Work for nine hours out of twelve	856	353	795	211

Upon the fasting-man Cetti, determinations of the respiratory exchange were made by Zuntz and Lehmann,[12] and they found that the absorption of oxygen and the discharge of carbon dioxide per kilo. of the man's weight quickly reached its minimum, and did not fall below this point during the progress of the fast; in fact there was a slight increase. Thus the absorption of oxygen per kilo. and minute was 4·65 c.c. on the third to sixth day, and 4·73 c.c. on the ninth to eleventh day of the fast. Before the first meal at the end of the

[1] See p. 719.

[2] "Œuvres," tome ii. pp. 695-696.

[3] "Die Verdauungssäfte und der Stoffwechsel," Leipzig, 1852, S. 321-362.

[4] *Ann. d. Chem. u. Pharm.*, 1862–63, Supp. Bd. ii. S. 52–361.

[5] *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1872, S. 1.

[6] *Landwirthsch. Versuchsstat.*, 1869, S. 306, 409.

[7] *Virchow's Archiv*, 1879, Bd. lxxvi. S. 136.

[8] *Arch. de biol.*, Gand, 1882, tome iii. p. 733.

[9] E. Smith, *Phil. Trans.*, London, 1859, vol. cxlix. p. 715; Hanriot and Richet, *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvi. p. 419; Zuntz, *Fortschr. d. Med.*, Berlin, 1887, Bd. v. S. 1; Meissel, Strohmer, and Lorenz, *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 63; Bœck and Bauer, *ibid.*, 1874, Bd. x. S. 336; Geppert, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 366; Hanriot and Richet, *Ann. de chim. et phys.*, Paris, 1891, Sér. 6, tome xxii. p. 495; Marcet, *Proc. Roy. Soc. London*, 1892, vol. l. p. 58; 1893, vol. lii. p. 213; Rubner, *Beitr. z. Physiol. Carl Ludwig z. s.* 70 *Geburtst.*, Leipzig, 1887, S. 259; Johansson, Landergren, Sondèn, and Tigerstedt, *Skandin. Arch. f. Physiol.*, Leipzig, 1896, Bd. vii. S. 29.

[10] *Ztschr. f. Biol.*, Munchen, 1866, Bd. ii. S. 459.

[11] For criticism of the determination of oxygen, see p. 696.

[12] *Berl. klin. Wchnschr.*, 1887, S. 428.

fast the absorption of oxygen and the discharge of carbon dioxide were 4·67 c.c. and 3·16 c.c. per kilo. and minute; after this meal the figures were respectively 5·05 c.c. and 3·46 c.c. The effects of the fast and of food upon the respiratory quotient were as follows :—

On last day of food, mixed diet . .	$\frac{CO_2}{O_2}$	=	0·73
On second day of fasting	,,	,,	0·68
On third day of fasting	,,	,,	0·65
During the remainder of the fast . .	,,	,,	0·66–0·68
When food, mixed diet, was again taken .	,,	,,	0·73–0·81

Regarding the influence of diet upon the respiratory quotient, it is only necessary here to state that an animal fed on a vegetable diet has a quotient closely approaching unity, for its chief food, the carbohydrates, contains enough oxygen to combine with the hydrogen to form water; that a carnivorous animal has a quotient about 0·74, and an omnivorous animal, such as man, a somewhat higher quotient[1]; and finally, that even a herbivorous animal has a low quotient during starvation, for it then lives upon its own tissues.

The influence of activity of the alimentary canal upon the respiratory exchange. — It has already been shown that a meal increases the respiratory exchange, and this effect was originally attributed solely to the oxidation of the food material taken up by the blood. Speck,[2] however, in 1874, pointed out that this increase in metabolism followed the taking of food so rapidly that it appeared to be due, in the first place, to the augmented activity of the alimentary canal. The first experiments to support this view were those made by Mering and Zuntz,[3] who showed that food placed in the stomach increased the absorption of oxygen and the discharge of carbon dioxide, whereas substances such as lactic acid, butyric acid, glycerin, sugar, egg albumin, and peptone, injected into the blood, increased the output of carbon dioxide, but had no marked effect upon the intake of oxygen. Rubner[4] and Fredericq[5] also found increased metabolism after food, due apparently, in the first place, to the activity of the glands of the alimentary canal;[6] and the observations made by Lehmann and Zuntz[7] upon the fasting-man Cetti showed that during the fast the respiratory exchange was constant, except on two days when Cetti suffered from colic; there was then an increase in the intake of oxygen and the output of carbon dioxide. These pieces of evidence have been followed up by Löwy,[8] who determined the respiratory exchange of fasting men before and after the activity of the alimentary canal had been increased by a dose of sodium sulphate, or a draught of cold water. Experiments made upon six men showed that the increased activity of the alimentary canal brought about in this way increased the intake of oxygen and the output of carbon dioxide by about 10 per cent.; the greatest increase

[1] See tables on pp. 706–708.
[2] *Arch. f. exper. Path. u. Pharm.*, Leipzig, 1874, Bd. ii.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xv. S. 634; 1883, Bd. xxxii. S. 173.
[4] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 330.
[5] *Arch. de biol.*, Gand, 1882, tome iv. p. 433.
[6] See also Slosse, *Arch. f. Physiol.*, Leipzig, 1890, Suppl. Bd. S. 164; Tangl, *ibid.*, 1894, S. 283.
[7] *Berl. klin. Wchnschr.*, 1887, S. 428.
[8] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. S. 515.

was about 24 per cent. in the carbon dioxide, and 17 per cent. in the oxygen. Sodium chloride and sodium bicarbonate had no effect on the intestines or upon the respiratory exchange. Löwy suggests that the therapeutic value of the waters at Carlsbad and Marienbad, in cases of disordered metabolism, may be partly due to this stimulating effect of sodium sulphate.

The influence of the size of the animal upon the respiratory exchange.[1]—The smaller an animal the greater is its surface in relation to its mass, for the surface increases as the square, the mass as the cube. Now, small mammals and birds have a temperature equal to or even higher than that of large animals of the same classes; and, on account of the relatively greater surface which they expose for the loss of heat, they must have a relatively far greater production of heat than the large animals, for there is generally no marked difference in the protective coat of fur or feathers. Heat is produced by a process of combustion in the tissues, and the respiratory exchange is a measure, although it may not be an absolutely exact one, of this combustion. Theoretically, therefore, a much more vigorous respiratory exchange should exist in the smaller warm-blooded animals. The experiments of many observers, especially of Letellier,[2] Regnault and Reiset,[3] Pott,[4] and Richet,[5] have shown that such is the case, not only for animals of the same species, living upon similar diet and having similar habits, but also for animals of different species, with very different diets and habits.[6]

Paul Bert[7] has shown that this difference in the rate of metabolism in small and large animals has become habitual, for it persists even when the animals are put under abnormal conditions of such a kind that the loss of heat is relatively the same; in such an experiment a pigeon absorbed 234 c.c. of oxygen per 100 grms. of its body weight, and a sparrow 467 c.c. of oxygen.

A series of experiments have been made by Richet[8] upon thirty-eight dogs of different sizes, their weights ranging from 2·2 to 28 kilos., and the results show that the output of carbon dioxide bears a very constant relation to the surface of the body, 0·0027 grms. per hour for each square centimetre of surface. A similar relation holds good for the intake of oxygen, the respiratory quotient being 0·748. This difference in metabolism is controlled by the nervous system, for it was found, in eighteen dogs of different sizes, anæsthetised with chloral, that the respiratory exchange was proportional to the weight of the body, 0·640 to 0·694 grms. CO_2 per kilo. and hour. A somewhat similar series of observations, made upon birds[9] of different sizes and species, gave similar results.

[1] For a discussion of this subject, see paper by Hoesslin, *Arch. f. Physiol.*, Leipzig, 1888, S. 323, where numerous references are given; Rubner, *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 535.

[2] *Ann. de chim. et phys.*, Paris, 1845, Sér. 3, tome xiii. p. 478.

[3] *Ibid.*, Paris, 1849, Sér. 3, tome xxvi. p. 299.

[4] *Landwirthsch. Versuchsstat.*, Bd. xviii. S. 81.

[5] *Arch. de physiol. norm. et path.*, Paris, 1890, tome xxii. pp. 17, 490; 1891, tome xxiii. p. 74.

[6] See tables, pp. 706–708.

[7] "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 503.

[8] *Compt. rend. Acad. d. sc.*, Paris, 1889, tome cix. p. 190; *Arch. de physiol. norm. et path.*, Paris, 1890, tome xxii. p. 17.

[9] *Arch. de physiol. norm. et path.*, Paris, 1890, tome xxii. p. 490.

The influence of time of day upon the respiratory exchange.—During a series of experiments performed in the years 1813 and 1814, Prout[1] discovered that the amount of carbon dioxide discharged from the lungs appeared to be influenced by the time of day, for there was a regular daily variation; the maximum was generally between 11 A.M. and 1 P.M.; then there was a fall to the minimum at 8 P.M. or 9 P.M. in the evening, and at this low level the discharge of carbon dioxide remained until 3 A.M. or 4 A.M., when there was a marked rise. A similar variation was observed by Vierordt,[2] both in the discharge of carbon dioxide and in the amount of air respired, and the cause of the rise he attributed to food. The table on p. 722 gives his average results.

Speck[3] determined the daily variation, both in the intake of oxygen and in the output of carbon dioxide, and showed the influence of food in producing the maximum.

Further experiments upon the daily variation in the respiratory exchange have been made by Berg[4] and Fredericq.[5] The following curve represents the daily variation observed by Fredericq in the oxygen absorbed by a man:—

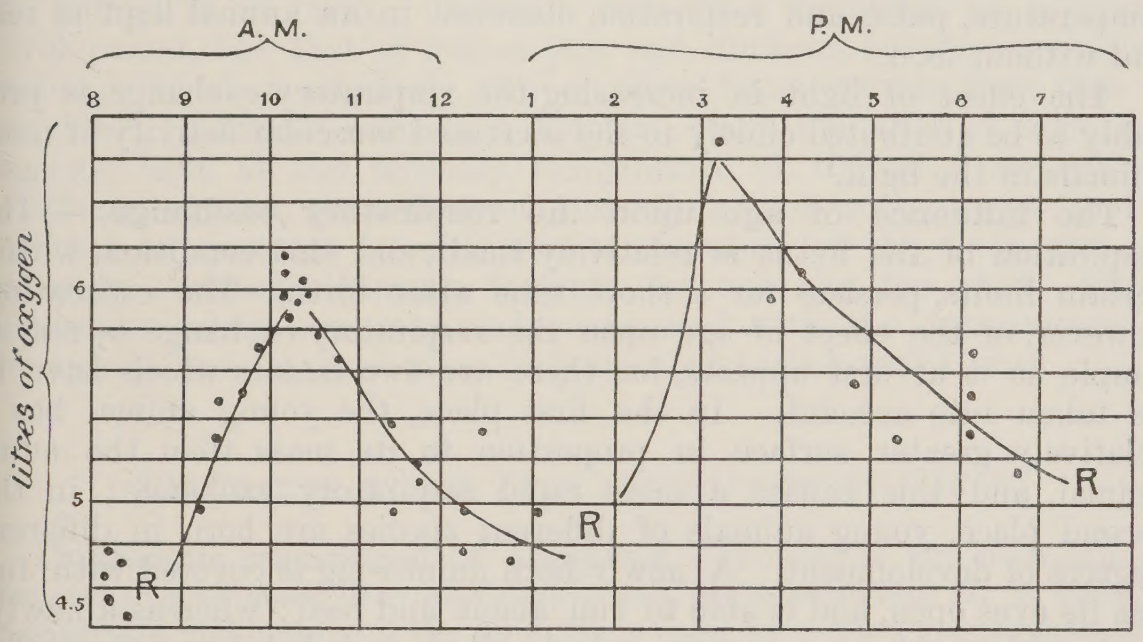


FIG. 67.—Fredericq's curve of daily variation in the absorption of oxygen.
R = time of a meal.

The causes of these variations in the daily respiratory exchange are to be attributed mainly to food and muscular activity, for during sleep, as E. Smith,[6] Pettenkofer and Voit,[7] and others[8] have shown, the metabolism is greatly diminished, and also in a less degree during hunger. In addition, however, there appears to be a certain periodicity stamped

[1] *Ann. Phil.*, London, 1813, vol. ii. p. 330; vol. iv. p. 331.

[2] "Physiol. d. Athmens," Karlsruhe, 1845; Wagner's "Handwörterbuch," Bd. ii. S. 883.

[3] "Untersuch. ueber Sauerstoffserbrauch u. Kohlensäureausathmung des Menschen," Cassel, 1871, S. 31; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1874, Bd. ii. S. 405; *ibid.*, 1880, Bd. xii. S. 1.

[4] "Einfluss der Athembewegungen auf die Ausscheidung der Kohlensäure durch die Lungen," Dorpat, 1869.

[5] *Arch. de biol.*, Gand, 1882, tome iii. p. 729.

[6] *Phil. Trans.*, London, 1859, vol. cxlix. p. 681.

[7] *Ztschr. f. Biol.*, München, 1866, Bd. ii. S. 459.

[8] Saint-Martin, *Compt. rend. Acad. d. sc.*, Paris, 1887, tome cv. p. 1124; Rubner, *Beitr. z. Physiol. Carl Ludwig z. s. 70 Geburtst.*, Leipzig, 1887, S. 259.

Hour of Day.	Respirations per Minute.	Volume of an Expiration.	Air expired in One Minute.	CO_2 expired in One Minute.	Percentage of CO_2 in the Expired Air.
		C.c. (37° and 758 Mm.).			
9 A.M. . .	12·1	503	6090	264	4·32
10 ,, . .	11·9	529	6295	282	4·47
11 ,, . .	11·4	534	6155	278	4·51
12 noon . .	11·5	496	5578	243	4·36
Dinner.					
1 P.M. . .	12·4	513	6343	276	4·35
2 ,, . .	13·0	516	6799	291	4·27
3 ,, . .	12·3	516	6377	279	4·37
4 ,, . .	12·2	517	6179	265	4·21
5 ,, . .	11·7	521	6096	252	4·13
6 ,, . .	11·6	496	5789	238	4·12
7 ,, . .	11·1	489	5428	229	4·22

upon the organism by long-continued habit—a day of work, a night of rest. This explains the persistence of the daily variation in metabolism, temperature, pulse, and respiration observed in an animal kept at rest and without food.

The effect of light in increasing the respiratory exchange is probably to be attributed chiefly to the increased muscular activity of most animals in the light.[1]

The influence of age upon the respiratory exchange. -- The respiration of the fœtus is relatively small, and this condition, within certain limits, persists for a short time after birth. The estimation, however, of the effect of age upon the respiratory exchange is not so simple as it at first appears, for there are two factors which have to be taken into account. In the first place, the young animal has a relatively greater surface in proportion to its mass than the adult animal, and this causes a more rapid respiratory exchange; in the second place, young animals of different species are born in different degrees of development. A newly-born guinea-pig is covered with fur, has its eyes open, and is able to run about and feed; whereas a newly-born rabbit, rat, or mouse is naked, blind, and helpless. A similar contrast is observed in the condition of the newly-hatched chick and pigeon. Elsewhere it has been shown that newly-born mammals and birds can be arranged in two classes—those which can and those which cannot maintain their temperature; and there is a similar contrast in the effect of changes of external temperature upon their respiratory exchange. These factors must, therefore, be remembered in estimating the effect of age upon the respiratory exchange.

In the case of man, experiments have been made by Andral and Gavarret,[2] but it is difficult to estimate the influence of the ratio between mass and surface of the body, for the weights of the subjects are not given. In the following table are some of Scharling's [3] results, which show that, weight for weight of body, the child discharges more carbon dioxide than the adult :—

[1] Numerous references to the controversy on this point will be found in a paper by Fubini and Benedicenti, *Arch. ital. de biol.*, Turin, 1891, vol. xvi. p. 80.

[2] "Recherches sur la quantité d'acide carbonique exhalé par le poumon dans l'espèce humaine," Paris, 1843. Extract in *Ann. de chim. et phys.*, Paris, 1843, Sér. 3, tome viii.

[3] *Ann. d. Chem. u. Pharm.*, 1843, Bd. xlv. S. 214.

Sex.	Age.	Weight.	Output of Carbon Dioxide in Twenty-four Hours.	Output of Carbon Dioxide per Kilo. and Hour.
		Kilo.	Grms.	Grms.
Male . .	35 years	65·5	804·72	0·512
,, . .	28 ,,	82	878·95	0·497
,, . .	16 ,,	57·75	822·69	0·594
Female . .	19 ,,	55·75	608·22	0·455
Male . .	9¾ ,,	22	488·14	0·925
Female . .	10 ,,	23	459·87	0·833

A series of experiments by Pembrey[1] has shown that the effect of age upon the respiratory exchange must be considered in relation to the temperature of the external air and the stage of development in which the animal is at birth. Animals born in a condition of advanced development, like that of guinea-pigs and chickens, have a respiratory exchange which is relatively two or three times greater than that of the adult. Animals born in a helpless state, like that of mice and pigeons, have at the ordinary temperature of the air a metabolism relatively smaller than that of the adult; but with a rise in the external temperature towards the temperature of the body the respiratory exchange increases towards the value in the adult. These differences, which are intimately connected with the temperature of the animal, are discussed more fully in other parts[2] of this work.

Respiration by the Skin and Alimentary Canal.

Cutaneous respiration of amphibia.—In many of the lower animals the exchange of gases between the skin and the surrounding air or water is considerable, and in some of the amphibia is equal to, or even greater than, that effected by the lungs. As early as the end of the last century, Spallanzani[3] showed that many amphibia could readily take up oxygen and discharge carbon dioxide after their lungs had been removed, and that in this condition they lived longer than animals of the same species whose skin had been covered with varnish. These observations were extended by Edwards,[4] who found that frogs deprived of their lungs would live a long time, provided that the external temperature was low. This cutaneous respiration took place as readily in flowing water as in air, for normal frogs could be kept alive although never allowed to come to the surface, provided that the temperature of the water did not exceed 12°; the cutaneous respiration was sufficient for the small amount of metabolism which occurred at low temperatures. Regnault and Reiset[5] found, by direct experiment, that frogs absorbed as much oxygen and discharged as much carbon dioxide after, as before, removal of their lungs. The following figures give their results:—

[1] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 363.
[2] See "Animal Heat," this Text-book, vol. i.
[3] "Mémoires sur la respiration," trad. par Senebier, Genève, 1803, pp. 72, 114.
[4] "De l'influence des agens physiques sur la vie," Paris, 1824, pp. 12, 41–62.
[5] *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi. p. 506.

Condition of Frog.	Oxygen per Kilo. and Hour.	Carbon Dioxide per Kilo. and Hour.	$\frac{CO_2}{O_2}$	Temperature.	Remarks.
	Grms.	Grms.			
Normal . .	Min. 0·063	0·045	·69	15°–9°	Result of five experiments.
	Max. 0·105	0·081	·78	,,	
After removal of lungs . .	0·047	0·049	·76	17°	...
	0·066	0·071	·79	21°	...

Berg,[1] on the other hand, found the discharge of carbon dioxide considerably diminished, but this has not been confirmed by Fubini,[2] who observed, in comparative experiments, only a slight decrease in the output of carbon dioxide after removal of the lungs.

To all of these experiments there are certain objections. The varnish, especially when containing alcohol, acts injuriously on the frog, and interferes with its free movement; the removal of the lungs, apart from the actual injury done to the animal during the operation, may cause the skin to take on vicariously the function of respiration. Later experiments by Klug[3] are free from these objections, for the head of a normal frog was passed through a rubber collar into one part of a chamber, while the body was retained in the other part—the pulmonary and cutaneous respiration were thus determined separately; and in order to allow for the cutaneous respiration which would take place on the head, other experiments were made, in which only the nose projected, and in which section of the vagi nerves, an operation which suspends the pulmonary respiration, had been performed. The results show that, during the winter at least, the cutaneous respiration is far more important than the pulmonary.

Experiment.	Duration of Experiment.	Weight of Frog.	Sex.	I.—CO_2 per 100 Grms. and 24 Hours. Head and Lungs.	II.—CO_2 per 100 Grms. and 24 Hours. Body below Head.	Ratio of I. to II.	Remarks.
	Hours.	Grms.		Grms.	Grms.		
1	3	77	Male	·0581	·1891	1–3·2	Normal.
3	,,	111	,,	·0540	·1902	1–3·5	,,
8	,,	82	Female	·0536	·2361	1–4·4	Only the nose projected through the partition.
9	,,	177	,,	·0175	·0786	1–4·4	Both vagi cut; membrane as in Experiment 8.

Dissard[4] has determined the production of carbon dioxide after ligature of the cutaneous or the pulmonary blood vessels of frogs, and he finds that both cutaneous and pulmonary respiration are necessary to the animal, for the

[1] "Untersuch. ueber d. Hautathmung d. Frosches," Diss., Dorpat, 1868.
[2] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1878, Bd. xii. S. 100.
[3] *Arch. f. Physiol.*, Leipzig, 1884, S. 183.
[4] *Compt. rend. Acad. d. sc.*, Paris, 1893, tome cxvi. p. 1153.

removal of either causes death after a longer or shorter period. The cutaneous respiration appears to be the more important during the winter, and the pulmonary during the summer. The experiments of Marcacci[1] indicate that the mucous membrane of the mouth and pharynx is also respiratory in the frog, and Camerano[2] finds in the case of the salamanders *Spelepes fuscus* and *Salamandrina perspicillata*, in which the lungs are either absent or rudimentary, that the bucco-pharyngeal respiration is more important than that carried on by the skin.

Valentin[3] determined the absorption of oxygen and the discharge of carbon dioxide from pieces of skin removed from the body of the frog, and found that the former process was the more active. This has been confirmed by Waymouth Reid and Hambly,[4] who, from experiments upon the transpiration through the frog's skin, conclude that there is no evidence of any physiological action by virtue of which carbon dioxide is "secreted"; the exchange of gases is the direct result of a difference of tension on the two sides of the respiratory septum.

Cutaneous respiration of mammals.—In man and other mammals the cutaneous respiration is so small that it has been denied by some observers,[5] and explained away by others, as arising from the decomposition of filth and cutaneous secretions.[6] Although Hippocrates and Galen believed in the absorption of air by the skin, no experiments appear to have been made until the year 1777, when Milly observed, during a warm bath, a number of small bubbles attached to the surface of his body; some of these bubbles were collected, and on analysis were found by Lavoisier[7] to be carbon dioxide. Objection was raised to this experiment, on the ground that carbon dioxide present in the water might attach itself to the body, as it does to other solid substances. Cruikshank,[8] however, found that air, in which a previously washed hand or foot had been confined for one hour, caused a marked turbidity with lime water. These experiments were extended by Abernethy,[9] who showed that in ordinary air oxygen was absorbed and carbon dioxide was given off as readily as in pure oxygen, whereas in carbon dioxide gas nitrogen was discharged and carbon dioxide absorbed by the skin of the hand.

In Lavoisier and Seguin's[10] experiments a man was enclosed in an air-tight rubber bag, while he breathed through two tubes connected with the mouth and nose; this method was improved by Scharling,[11] who prevented the excessive accumulation of moisture by ventilating the chamber in which the subject of the experiment was confined. The results of the above and later observers are given in the following table:—

[1] *Arch. ital. de biol.*, Turin, vol. xxi. p. 1.
[2] *Ibid.*, vol. xxi. p. 387.
[3] *Arch. f. physiol. Heilk.*, Stuttgart, 1855, S. 474.
[4] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 411.
[5] Priestley, "On Air," vol. ii. pp. 193, 194; Klapp and Gordon, "Ellis's Inquiry" Edinburgh, 1807, pp. 189, 354.
[6] Hoppe-Seyler, "Physiol. Chem.," Berlin, 1879, Bd. iii. S. 580.
[7] *Hist. Acad. roy. d. sc.*, Paris, 1777, pp. 221, 360.
[8] "Experiments on the Insensible Perspiration of the Human Body, showing its affinity to Respiration," 2nd edition, London, 1795, pp. 81, 82.
[9] "Surgical and Physiological Essays," London, 1793, pt. 2, p. 107.
[10] "Œuvres de Lavoisier," Paris, 1862, tome ii. p. 708; *Ann. de chim. et phys.*, Paris, 1814, tome xc. p. 8.
[11] *Journ. f. prakt. Chem.*, Leipzig, 1845, Bd. xxxvi. S. 454; *Ann. de chim. et phys.*, Paris, 1843, Sér. 3, tome viii. p. 480.

Part of Body Examined.	Gases Discharged.	Gases Absorbed.	CO_2 Discharged. For Total Surface of Body in 24 hours.	O_2 Absorbed. For Total Surface of Body in 24 hours.	Observer.
Hand and foot of man	Carbon dioxide	Not determined	...	...	Cruikshank.[1]
Hand of man	Carbon dioxide, and sometimes nitrogen	Oxygen	14 grms.	...	Abernethy.[2]
Total surface of skin of man	...	,,	...	...	Lavoisier and Seguin.[3]
Portions of skin of man	Carbon dioxide and nitrogen	Not determined	...	...	Collard de Martigny.[4]
Total surface of skin of man	Carbon dioxide	,, ,,	32·8 grms.	...	Scharling.[5]
Total surface of skin of child, æt. 10	Carbon dioxide	,, ,,	10·9 grms.	...	,,
Total surface of skin of girl æt. 19	,, ,,	,, ,,	23·9 grms.	...	,,
...	,, ,,	,, ,,	,,	...	Regnault and Reiset.[6]
Portion of skin of man	,, ,,	Oxygen	8·4 grms.	2·7 grms.	Gerlach.[7]
Arm of man	,, ,,	Not determined	2·2 grms.	,,	Reinhard.[8]
,,	,, ,,	,, ,,	14 grms.	,,	Röhrig.[9]
Total surface of skin of man, except head	,, ,,	,, ,,	6·3 grms. (maximum)	,,	Aubert and Lange.[10]
,,	,, ,,	,, ,,	2·3 grms. (minimum)	,,	,, ,,
Hand . . .	,, ,,	,, ,,	1·25 grms.	,,	,, ,,
Hand and forearm	,, ,,	,, ,,	6·80 grms.	,,	Fubini and Ronchi.[11]
Upper limb of man	,, ,,	,, ,,	·0193 grm.[12]		Barratt.[13]
Portion of skin of a horse	,, ,,	Oxygen	30·1 grms.	6·3 grms.	Gerlach.[7]
Total skin of a horse	,, ,,	,,	119 grms.	,,	Zuntz, Lehmann, and Hagemann.[14]

The results of Aubert which have been given above show that the cutaneous respiration varies in intensity in different parts of the body, and that for this reason it is impossible to correctly calculate the cutaneous respiration of the whole body from the data obtained on one limited part, such as the hand. Further, the exchange of gases from the

[1] *Loc. cit.* [2] *Loc. cit.*

[3] "Œuvres de Lavoisier," Paris, 1862, tome ii. p. 708; *Ann. de chim.*, Paris, 1814, tome xc. p. 8.

[4] *Journ. de physiol. expér.*, Paris, 1830, tome x. p. 162.

[5] *Journ. f. prakt. Chem.*, Leipzig, 1845, Bd. xxxvi. S. 454, *Ann. de chim. et phys.*, Paris, 1843, Sér. 3, tome viii. p. 480.

[6] "Recherches sur la respiration des animaux," p. 209.

[7] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 431.

[8] *Ztschr. f. Biol.*, München, 1869, Bd. v. S. 28.

[9] *Deutsche Klinik*, Berlin, 1872, Bd. xxiv. S. 209, 225, 234.

[10] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 539.

[11] *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1881, Bd. xii. S. 1.

[12] For upper limb alone and for one hour; temperature of air = 35°.

[13] *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 204.

[14] *Arch. f. Physiol.*, Leipzig, 1894, S. 351.

skin is increased by exercise, a rise of temperature, and by any cause which produces increased vascularity of the skin, such as friction, warm baths, and electric shocks.[1] It is also said to be influenced by food and by exposure to light.[2]

The experiments of Gerlach, Röhrig, and others show that the skin of animals will absorb carbon dioxide, carbon monoxide, sulphuretted hydrogen, and the vapour of chloroform and ether.

The effects of varnishing the skin.—The old theory, held by Galen, Sanctorius, and others, that many diseases were due to the retention of waste substances which in a normal condition would have been discharged from the body, received great support from experiments in which the skin of animals had been covered by an impermeable layer of varnish or ointment. At the same time it was held that the results showed the imperative necessity of cutaneous respiration and perspiration. The symptoms observed after the skin of an animal was varnished were restlessness, shivering, increased rapidity of breathing and heart-beat, soon followed by slow respiration and pulse, a fall in temperature to 20° or 19°, the discharge of albumin in the urine, spasms, and death. Examination of the body after death showed congestion of the skin, subcutaneous tissue, muscles, and internal organs.[3]

The earliest experiments appear to have been made by Fourcault,[4] Ducros,[5] Becquerel and Brechet,[6] Gluge,[7] and Magendie.[8] The temperature was observed by Gerlach,[9] who obtained the following results for a rabbit and a horse, after their skins had been covered with a layer of linseed oil:—

Animal.	Temperature Before.		Temperature After.		Remarks.
	Rectal.	Cutaneous.	Rectal.	Cutaneous.	
Rabbit . .	39°·7	38°	28°	26°	At time of death, thirty hours after varnishing.
Horse . . .	38°	35°	32°	29°	On the sixth day after varnishing. Death on eighth day.

Edenhuizen[10] showed that death followed even when only one-sixth of the total cutaneous surface was varnished; he believed that the symptoms were due to an alkali which he found in the skin. A further advance in knowledge was made when Valentin[11] discovered that the discharge of carbon dioxide from the lungs was reduced to one-eighth or

[1] Gerlach, Aubert, Röhrig, Barratt, *loc. cit.*
[2] Fubini and Ronchi, *loc. cit.* Here other references will be found.
[3] Valentin, *Arch. f. physiol. Heilk.*, Stuttgart, Bd. xi. S. 433.
[4] *Compt. rend. Acad. d. sc.*, Paris, Mars 16, 1837.
[5] *Notiz. a. d. Geb. d. Nat.-u. Heilk.*, Weimar, 1841, Bd. xix.
[6] *Arch. gén. de méd.*, Paris, 1841, tome xii. p. 517.
[7] *Abhandl. z. Physiol. u. Path.*, Jena, 1841, S. 66.
[8] *Gaz. méd. de Paris*, Dec. 6, 1843.
[9] *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1851, S. 431.
[10] *Nachr. v. d. k. Gesellsch. d. Wissensch. u. d. Georg.-Aug. Univ.*, Göttingen," 1861, S. 288.
[11] *Arch. f. physiol. Heilk.*, Stuttgart, Bd. ii. S. 433.

one-sixth of the normal amount, but that the output of carbon dioxide was raised to the normal, and death was prevented, when the temperature of the surroundings was kept at 20°–25°. These observations were confirmed by Schiff.

The explanation, however, of these experiments was given in 1868, when Laschkewitsch [1] showed by calorimetric observations that varnished animals gave off an abnormally large quantity of heat, that the cutaneous vessels were dilated and the vasomotor nerves appeared to be paralysed, that the temperature of the animal fell, and thus caused the characteristic symptoms and death. When only one limb of a rabbit was varnished, the temperature under the skin of that part was 34°·5, as compared with 33°, that of the normal limb; after one hour, the first fell to 33°·2, the second to 32°·5. Varnished animals wrapped up in cotton-wool remained well, and no bad effect was observed when the body of a normal rabbit was enclosed for six hours in a cylinder filled with hydrogen, the rabbit breathing through a mask over the nose and mouth. Laschkewitsch also pointed out that the greater the surface of the skin in relation to the mass of the body, the sooner death followed varnishing of the skin. This is shown in the experiments which Gerlach made upon rabbits and horses, the former dying in thirty hours, the latter after seven or eight days. The greater the surface in relation to the mass of the body, the greater is the rate of cooling.

The experiment of varnishing the human body was first made, according to Laschkewitsch, by the officials of Pope Leo X., who, wishing during the coronation ceremonies to make a child represent an angel, gilded the whole of its body; the child, however, died before it had fulfilled its part in the ceremony. It is probable that in this case the gilding contained some poisonous substance. In 1877, Senator [2] showed that the whole surface of the human body could be covered with an impermeable layer, and that even after remaining in this condition for eight or ten days, no disturbance whatever could be observed; no marked change was observed in the temperature, and this explains the absence of the symptoms which are observed in animals. The human body has little natural covering and the most perfect power of regulating its temperature, conditions which do not obtain in most of the lower animals.

Extensive but superficial burns of the skin often cause death, and this, according to some observers, is due to interference with the cutaneous respiration and to retention of waste products, which are normally discharged by the sweat. There is, however, very little evidence in support of this view, and it is probable that the fatal result in these cases is due to the following factors—shock, changes in the plasma and corpuscles of the blood,[3] excessive loss of heat from the hyperæmic skin, and disturbed regulation of temperature, owing to the absence of the normal sensory impulses from the skin.

Respiration in the alimentary canal.—The quantity and nature of the gases found in the alimentary canal vary under different circum-

[1] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1868, S. 61.

[2] *Virchow's Archiv*, 1877, Bd. lxx. S. 182; *Arch. f. Physiol.*, Leipzig, 1894, S. 178.

[3] Max Schultze, *Arch. f. mikr. Anat.*, Bonn, 1865, Bd. i. S. 26; Wertheim, *Wien. med. Presse*, 1868, No. 13; Ponfick, *Berl. klin. Wchnschr.*, 1877, No. 46; *Centralbl. f. d. med. Wissensch.*, Berlin, 1880, Nos. 11 and 16; Lesser, *Virchow's Archiv*, 1880, Bd. cxxix. S. 248; Hoppe-Seyler, *Ztschr. f. physiol. Chem.*, Strassburg, 1881, Bd. v., S. 1 and 344; Tappeiner, *Centralbl. f. d. med. Wissensch.*, Berlin, 1881, Bd. xix. S. 385 and 401.

stances, as is shown by the following table, which gives the results obtained by Ruge[1] from the analysis of the gas obtained from the rectum of the same man under different conditions:—

Gas.	Milk Diet.	Vegetable Diet for Four Days.	Animal Diet for Three Days.
Oxygen	...	...	...
Nitrogen . . .	36·71	18·96	64·41
Hydrogen . . .	54·23	4·03	0·69
Marsh-gas . . .	...	55·94	26·45
Carbon dioxide . .	9·06	21·05	8·45
Hydrogen-sulphide .	...	Trace.	...

The distribution of these gases in the different parts of the alimentary canal was examined by Tappeiner,[2] in the body of a criminal, who had been executed a short time before the examination was made. The following are the results:—

Gas.	Stomach.	Ileum.	Colon.	Rectum.
Oxygen . .	9·19	67·71	...	...
Nitrogen . .	74·26		7·46	62·76
Hydrogen . .	0·08	3·89	0·46	...
Marsh-gas . .	0·16	...	0·06	0·90
Carbon dioxide .	16·31	28·40	91·92	36·40

Zuntz, Lehmann, and Hagemann[3] found in the gas drawn off from the intestine of a living horse about 22 per cent. carbon dioxide, 59 per cent. marsh-gas, and 2·5 per cent. hydrogen.

These gases have several sources of origin. Oxygen and nitrogen occur in the air swallowed; hydrogen, marsh-gas, and carbon dioxide are formed by the fermentations which take place in the contents of the alimentary canal; nitrogen and carbon dioxide, under certain conditions, diffuse from the tissues into the intestines, and carbon dioxide arises from the neutralisation of the sodium carbonate of the intestinal secretions. Further details on the origin of these gases will be found elsewhere;[4] here it is necessary only to consider the part

[1] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xliv. S. 739.

[2] *Arb. a. d. path. Inst. zu München*, Stuttgart, 1886, Bd. i. S. 226. See also Planer, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1860, Bd. xlii. S. 307; Hofmann, *Wien. med. Wchnschr.*, 1872: Tappeiner, *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 432; *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 228; 1884, Bd. xx. S. 52; *Arb. a. d. path. Inst. zu München*, Stuttgart, 1886, Bd. i. S. 215.

[3] *Arch. f. Physiol.*, Leipzig, 1894, S. 354.

[4] See "Chemistry of Digestion," this Text-book, vol. i.

some of them play in respiration. The oxygen in the air swallowed is almost entirely absorbed in the stomach; the carbon dioxide is generally 20 to 90 per cent. of the gas present in the intestines, and will therefore have a partial pressure greater than that of the carbon dioxide in the blood and tissues, and will diffuse from the intestines into the blood, to be ultimately discharged in the lungs. As regards the nitrogen, the quantity present in the alimentary canal is considerable, but its partial pressure is generally below that of the atmosphere, and of the tissues, and under these conditions there will be a diffusion of nitrogen from the blood and tissues into the intestinal tract. It is important to remember the presence of nitrogen and marsh-gas in the alimentary canal, for thus it is possible to explain those cases in which an absorption or discharge of nitrogen has been observed during determinations of the respiratory exchange. When carbon dioxide or hydrogen-sulphide is injected into the rectum, a portion of the gas is absorbed and excreted by the lungs.[1]

Paul Bert[2] observed that a kitten with ligatured trachea lived twenty-one minutes when a current of air was passed through the alimentary canal, whereas a kitten of similar age died in thirteen minutes, when the only operation performed was ligature of the trachea. A similar absorption of oxygen from the alimentary canal probably takes place in man under special circumstances; for swimmers who can remain under water for an exceptional length of time, state that they swallow air in addition to taking a deep inspiration before a dive.

In warm-blooded animals the alimentary canal plays an unimportant part in respiration, but this is not the case in some fish, for all the members of the loach family respire partly by the alimentary canal. The air discharged under normal conditions from the rectum of *Cobitis fossilis* has the following composition: 87·18 per cent. nitrogen, 12·03 per cent. oxygen, and 0·79 per cent. carbon dioxide; but if the fish be prevented from swallowing air for several hours, the percentage composition is 91·33 nitrogen, 7·94 oxygen, and 0·73 carbon dioxide.[3] Erman[4] opened the abdomen of one of these fish, and noticed that when air was swallowed the intestinal veins and the liver became bright red, but with hydrogen or nitrogen the colour was very dark purple. The mucous membrane of the intestine of *Cobitis fossilis* is, according to Leydig,[5] composed almost entirely of capillary blood vessels, and a little connective tissue. In the *Callichthys asper*, a fish found in Brazil, the respiration by the alimentary canal is essential for life, for if the fish be prevented from coming to the surface of the water to swallow air, it dies within two hours. The air discharged by the rectum contains 1·5–3·8 per cent. of carbon dioxide.[6]

The respiration of the fœtus.—The respiration of the fœtus was first understood and described in 1674 by Mayow,[7] who in his treatise, "De Respiratione Fœtus in Utero," maintains that the placenta is to be looked upon as a lung, from which the umbilical vessels take up the nitro-aerial gas

[1] Bernard, "Leçons sur les effets des substances toxiques et médicamenteuses," Paris, 1857, p. 59; Bergeon, *Compt. rend. Acad. d. sc.*, Paris, tome civ. p. 1812; Hanriot and Richet, *Compt. rend. Soc. de biol.*, Paris, 1887, p. 307; Flint, *Med. News*, Phila., 1887, vol. li. p. 670.

[2] "Physiol. comp. de la respiration," Paris, 1870, p. 173.

[3] Baumert, "Chem. Untersuch. ü. d. Respir. d. Schlammpeitzgers," Breslau, 1855, S. 24.

[4] *Ann. d. Phys. u. Chem.*, Leipzig, 1808, Bd. xxx. S. 113.

[5] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1853, S. 3.

[6] Jobert, *Ann. d. sc. nat.*, Paris, 1877, Zool. (6), tome v., Art. No. 8.

[7] "Tractatus Tertius, de Respiratione Fœtus in Utero et Ovo," Oxon., 1674.

(oxygen) and carry it to the fœtus; at the same time, he recognises that the fœtus obtains its supply of nutrition in a similar manner. This view of the fœtal respiration was adopted and extended by Hulse,[1] and by Ray,[2] who states his view in the following words:—"The maternal blood which flows to the cotyledons, and encircles the papillæ, communicates by them to the blood of the fœtus the air wherewith itself is impregnate; as the water flowing about the carneous radii of the fish's gills doth the air that is lodged therein to them." Mayow's brilliant work was allowed to drop into obscurity, and the respiration of the fœtus was not understood again until the beginning of this century.

Some physiologists, and among them Leclarc[3] and Geoffroy St. Hilaire,[4] maintained that the liquor amnii served the purpose of respiration by the skin of the fœtus. Haller,[5] Hunter,[6] Osiander,[7] Autenrieth and Schütz,[8] Emmert,[9] Joh. Müller,[10] and E. H. Weber[11] stated that no difference could be observed in the colour of the blood of the umbilical arteries and vein; on the other hand, Scheel,[12] Herissant and Diest,[13] Baudelocque,[14] Jœrg,[15] Jeffray,[16] and Bostock[17] noticed that the blood going from the placenta to the fœtus was of a more arterial hue than that going in the opposite direction, although there was naturally not so marked a distinction as between the arterial and venous blood of the adult.

Even as late as 1840 the respiration of the fœtus was not understood, for Joh. Müller,[18] the chief physiologist of the time, held that plasma from the mother passed to the fœtus, and so supplied the place of respiration. Bischoff[19] looked upon the placenta as an organ of the mother, and denied the existence of any special respiration; this view was contested by Litzmann,[20] who held that the fœtus respired by the placenta. Gradually, owing in a great measure to the work of Schwartz,[21] Gusserow,[22] and Schultze,[23] the truth discovered by Mayow in 1674 was re-established, and received a final proof when Zweifel,[24] following the suggestion of Hoppe-Seyler, showed in 1876 that the spectrum of oxyhæmoglobin could be clearly seen in the umbilical cord before the child breathed by its lungs; that, by taking the precaution to open the uterus of a pregnant rabbit in warm normal saline solution, and thus

[1] Quoted from Ray's book, p. 73.
[2] "The Wisdom of God in the Creation," 12th edition, 1759, p. 74.
[3] [4] [5] [6] [7] Quoted from Müller, "Elements of Physiology," Baly's transl., 1838, vol. i. pp. 317, 320.
[8] "Experimenta circa calorem fœtus sanguinem ipsius instituta," Tubingæ, 1799.
[9] *Arch. f. d. Physiol.*, Halle, 1811, Bd. x. S. 122.
[10] "De respiratione fœtus," Lipsiæ, 1823, S. 10; "Handbuch der Physiologie," 1840, Bd. ii. S. 729.
[11] Hildebrandt's "Anatomie," Bd. iv. S. 524.
[12] "De liquoris amnii asperæ arteriæ fœtuum humanorum natura et usu," Hafniæ, 1799.
[13] Haller's "Disputationes," vol. v. pp. 516, 526.
[14] Bichat's "Anatomie générale," tome ii. p. 465.
[15] "Die Zeugung," Leipzig, 1815, S. 273.
[16] "De Placenta."
[17] "Physiology," London, 1828, 2nd edition, vol. ii. p. 199.
[18] "Handbuch der Physiologie," 1840, Bd. ii. S. 729. His words are:—"Die von den Blutgefässen angezogenen Säfte dringen sodann direct ins Blut des Fötus. Durch diese Art von Wechselwirkung mit mütterlichen Säften ist bei dem Fœtus auch das Athmen ersetzt oder ein Æquivalent dafür gegeben."
[19] "Entwickelungsgeschichte der Säugethiere und des Menschen," 1842, S. 541.
[20] "Ueber die Schwangerschaft," Wagner's "Handwörterbuch."
[21] "Die vorzeitigen Athembewegungen," Leipzig, 1858.
[22] *Arch. f. Gynaek.*, Berlin, Bd. iii.
[23] *Jenaische Ztschr. f. Med. u. Naturw.*, Leipzig, Bd. iv.
[24] *Arch. f. Gynaek.*, Berlin, 1876, Bd. ix. S. 291.

prevent vigorous contractions of the uterus, the blood in the umbilical vein of the fœtus was brighter than that in the arteries; and that the difference in colour of the umbilical vein and arteries disappeared during asphyxia of the mother, to reappear when artificial respiration was performed. Pflüger[1] had also noticed that the colour of the umbilical vein was reddish brown in the normal condition, but became black during asphyxia.

The results obtained by Zweifel were confirmed and extended by Zuntz,[2] who showed that during asphyxia of the mother the fœtal blood lost oxygen in the placenta, the blood of the umbilical vein becoming darker than that of the corresponding arteries; that when the maternal vessels supplying the placenta were compressed the umbilical vein became as dark as the arteries; that a fœtus respiring air through its lungs lost oxygen in the placenta, which was left connected with an excised piece of the uterus; that during normal breathing of the mother the umbilical vein coming from the intact placenta contained blood as red as the arterial blood of the uterus, and that movements of the fœtus made the blood of the umbilical arteries darker in colour. Zuntz maintains that the oxidation taking place in the fœtus must be small, for the difference in the colours of the umbilical arteries and vein is slight, corresponding to a difference of about 1 per cent. in the amount of oxygen; and the fœtus can live for a long time upon the oxygen in its blood, when respiration by the placenta or lungs is prevented. According to Zuntz's estimate, the human fœtus would need daily 0·169 grm. of oxygen per kilo. of its weight, as compared with 14–15 grms., the amount required by an adult.[3] Pflüger[4] and Zuntz found that the blood of the fœtus, in comparison with that of an adult, had a low specific gravity and was poor in corpuscles and hæmoglobin; these results, however, are opposed to those of Hayem,[5] Hœsslin,[6] Sorensen,[7] Wiskemann,[8] Preyer,[9] Denis,[10] and others,[11] who found higher values for the fœtus than for the mother.

The difference in the tension of oxygen in the blood of the umbilical artery of the fœtus and the maternal blood is small, but it is sufficient, owing to the intimate relationship of the maternal and fœtal circulations, to supply the oxygen needed by the fœtus.[12]

Cohnstein and Zuntz[13] have analysed the blood of the umbilical artery of a fœtal sheep, which was 53 cm. long, weighed 1535 grms.,

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 80.
[2] *Ibid.*, Bonn, 1877, Bd. xiv. S. 605.
[3] This is contested by Wiener, *Arch. f. Gynaek.*, Berlin, 1884, Bd. xxiii. S. 183. This paper gives numerous references to the work on the general metabolism of the fœtus, but does not disprove the relatively small oxidation in the fœtus.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 61; 1875, Bd. x. S. 274.
[5] *Compt. rend. Acad. d. sc.*, Paris, 1877, tome lxxxiv. p. 1166.
[6] *Ztschr. f. Biol.*, München, 1882, Bd. xviii. S. 612.
[7] *Jahresb. ü. d. Fortschr. d. Anat. u. Physiol.*, Leipzig, 1878, Bd. v. Abth. 3, S. 192.
[8] *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 434.
[9] "Specielle Physiol. des Embryo," Leipzig, 1883, S. 144.
[10] *Ann. de chim. et phys.*, Paris, 1842, Sér. 3, tome v. p. 313.
[11] Poggiale, *Compt. rend. Acad. d. sc.*, Paris, 1847, tome xxv. p. 112; Panum, *Virchow's Archiv*, Bd. xxix. S. 481. See also Cohnstein and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiv. S. 183.
[12] Zuntz, *ibid.*, 1877, Bd. xiv. S. 626.
[13] *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiv. S. 206, 231.

and was probably in the last three weeks of intra-uterine life. The result was—

Oxygen	6·669	volumes	per cent.
Carbon dioxide	46·542	,,	,,
Nitrogen	1·000	,,	,,
Total gas	54·211	,,	,,

Comparative estimations of the gases in the umbilical artery and vein were also made, and show that the changes undergone by the blood in the placenta are about one-half as marked as in the lungs of an adult :—

		Oxygen.	Carbon Dioxide.	
Fœtal Sheep	Artery	6·69 vol. p.c.	45·54 vol. p.c.	Specimens of blood taken simultaneously.
	Vein	less than 11·36 ,,	41·82 ,,	
	Difference	4·67 ,,	4·72 ,,	
	Artery	2·3 ,,	47·0 ,,	Sample of blood from vein taken 24 minutes after that from artery.
	Vein	6·3 ,,	40·5 ,,	
	Difference	4·0 ,,	6·5 ,,	
Adult animals [1]	Difference between venous and arterial blood	8·15 vol. p.c.	9·2 vol. p.c.	

From these results Cohnstein and Zuntz calculate that the absorption of oxygen by a fœtal sheep weighing 3600 grms. is 1·75 c.c. per minute, or, per kilo. and minute, 0·49 c.c., which is about one-twelfth the amount absorbed, weight for weight of body, by a full-grown sheep.

The respiration of the embryo.—The process of respiration in the embryo has, owing to the natural difficulties of the subject, been chiefly studied in the eggs of birds and of a few reptiles. The absorption of nitro-aerial gas (oxygen) through the porous shell of an egg undergoing incubation appears to have been first recognised by Mayow,[2] but the necessity of respiration in the developing embryo was first shown by the experiments [3] of varnishing the eggs, covering them with oil or warm water ; under such conditions it was found that the embryo quickly ceased to develop, and died. If the impervious covering was only applied to a portion of the shell, the embryo developed, in some cases normally, in others abnormally with the production of deformities or monstrosities.[4]

[1] Zuntz, Hermann's "Handbuch," Bd. iv. Th. 2, S. 37.

[2] "Tract. quinque," Oxonii, 1674, pp. 131, 313, 321.

[3] Paris, *Ann. Phil.*, London, 1821, N.S., vol. ii. p. 2 ; Home, *Phil. Trans.*, London, 1810, p. 213 ; 1822, p. 339 ; Dareste, *Ann. d. sc. nat.*, Paris, 1855, Sér. 4, Zool., tome iv. p. 119 ; *Compt. rend. Acad. d. sc.*, Paris, 1855, p. 963 ; Marshall, *Med. Times and Gaz.*, London, 1840–41, vol. i. p. 242 ; Dusing, *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiii. S. 67. Here other references are given.

[4] Gerlach and Koch, *Biol. Centralbl.*, Erlangen, 1882, Bd. ii. S. 681.

In 1834, Theodor Schwann [1] showed that, when hens' eggs are kept at a warm temperature in gases containing no oxygen, the germinal membrane enlarges, and the area pellucida is formed, but no embryo ; eggs would develop normally in warm air, after they had been in hydrogen for twenty-four hours at a warm temperature, but not if the exposure to hydrogen had lasted thirty hours or more.

The first determinations of the respiratory exchange in eggs are due to Baudrimont and Martin Saint-Anges,[2] who showed that the eggs of birds and of snakes gave off carbon dioxide during incubation, and that the embryos of frogs died if placed in water free from air. The quantitative results obtained by these observers are not trustworthy, owing to the defective methods of gas analysis then in use. The first reliable determinations are those made by Baumgärtner [3] throughout the period of incubation of hens' eggs. The following table gives some of the results :—

Day of Incubation.	Loss of Weight of Egg.		Discharge of Carbon Dioxide.		Absorption of Oxygen.	
	From the commencement of incubation.	On the day in question.	For one egg.	For one kilo. of eggs.	For one egg.	For one kilo. of eggs.
	Grms.	Grms.	Grms.	Grms.	Grms.	Grms.
1	...	0·125	0·009	0·16	0·0074	0·13
9	1·853	0·164	0·048	1·01	0·0360	0·76
20	10·479	0·212	0·560	18·93	0·4435	14·90
21 (chick free)	...	...	1·008	...	0·7317	...

Similar experiments were made by Pott and Preyer,[4] who found that a fertile egg, weighing 50 grms., lost in weight about 10·27 grms. during incubation, an unfertile one 9·70 grms., and an egg kept at the temperature of an ordinary room 1·66 grms., in twenty-one days. The respiratory exchange of a developing embryo in an egg weighing 50 grms. was, for periods of twenty-four hours :—

Day of Incubation.	Discharge of Carbon Dioxide.	Absorption of Oxygen.
	Grms.	Grms.
7	0·09	0·09
13	0·24	0·24
21	0·86	0·68

Pott [5] also showed that the development of the embryo is not hastened or delayed if the egg is incubated in an atmosphere of oxygen. During incubation, it has been proved that the temperature of the embryo, owing to its metabolism, is slightly warmer than the temperature of its surroundings.[6]

[1] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1835, S. 121.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1843, tome xvii. p. 1343 ; *Ann. de chim. et phys.*, Paris, 1847, Sér. 3, tome xxi. p. 195.

[3] "Der Athmungsprozess im Ei," Freiburg im B., 1861.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxvii. S. 320.

[5] *Ibid.*, 1883, Bd. xxxi. S. 268.

[6] Bärensprung, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 126. See also "Animal Heat," this Text-book, vol. i.

In connection with the respiration of the embryo chick, it is interesting to find that the air contained in the air chamber of the egg has been stated to have a greater percentage of oxygen than that present in the atmosphere. Thus Bischof [1] found 23·475 volumes per cent. as the mean of four analyses, and Dulk [2] obtained in one case 25·26, in another case 26·77 per cent. of oxygen. Hüfner,[3] however, has repeated and extended these observations, and found the following composition in the air removed from twelve eggs, unincubated, and a few weeks old:—Oxygen 18·94, nitrogen 79·97, and carbon dioxide 1·09 volumes per cent.; and in the case of two goose eggs, incubated for sixteen days, oxygen 19·58 and 19·85, nitrogen 79·55 and 78·62, carbon dioxide 0·87 and 1·53 volumes per cent.; these eggs showed no trace of an embryo. Experiments were also made upon the rate of diffusion of gases through the egg-shell and the shell-membrane, and it was found that the rates of diffusion of the different gases did not follow Graham's law; they were not inversely proportional to the square roots of the densities of the several gases.

During the period of incubation of a chick the gradual development of the power of heat regulation can be traced. At first the embryo responds to changes in external temperature by a similar change in its respiratory exchange—a fall of temperature causes a decrease, a rise of temperature an increase, in the respiratory exchange; then for a short time there is an intermediate condition in which a change of temperature has no marked effect; and, lastly, when the chick is hatched, it responds as a warm-blooded animal.[4]

If tadpoles and larvæ of salamanders (*Salamandra maculata*) be prevented from coming to the surface of the water, their metamorphosis is greatly prolonged, and if well fed they will live for a long time as purely aquatic animals.[5]

The Respiration of Different Gases.

Some gases, such as hydrogen and nitrogen, have no specific effect when they are respired, and animals supplied with these gases alone die simply from want of oxygen. Other gases, such as carbon dioxide, carbon monoxide, nitrous oxide, and hydrogen sulphide, can be taken into the lungs, and if present in sufficient quantity are absorbed, and produce specific effects; while a third class, such as ammonia and nitric oxide, are irrespirable on account of their irritant action producing spasm of the glottis.

Oxygen.—Soon after his re-discovery [6] of oxygen in 1774, Priestley [7] observed, both upon himself and upon animals, the effect of breathing the pure gas; in his own case he felt an agreeable facility of respiration, and in animals he found that oxygen had a greater power than air in supporting life. These experiments were repeated by Lavoisier,[8] Higgins,[9] Dumas,[10] Beddoes,[11] H. Davy,[12] Allen and Pepys,[13] and in some

[1] *Journ. f. Chem. u. Phys.*, Nürnberg, 1823, Bd. xxx. S. 446.
[2] *Ibid.*, Halle, 1830, Bd. lviii. S. 363.
[3] *Arch. f. Physiol.*, Leipzig, 1892, S. 467.
[4] Pembrey, Gordon, and Warren, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvii. p. 331; Pembrey, *ibid.*, 1895, vol. xviii. p. 361.
[5] Preyer, "Specielle Physiologie des Embryo," Leipzig, 1885.
[6] Mayow can rightly claim to have discovered oxygen before 1674. See his "Tractatus quinque."
[7] "On Air," vol. ii. p. 162.
[8] *Mém. Soc. Roy. Med.*, 1782, tome iii. p. 576; *Hist. Acad. roy. d. sc.*, Paris, 1789, p. 573.
[9] "Minutes of a Society, etc.," London, 1795, p. 144.
[10] "Physiologie," Paris, 1806, 2nd edition, tome iii. p. 59.
[11] "On Factitious Airs," Bristol, 1796, part i. p. 13.
[12] "Researches," p. 439.
[13] *Phil. Trans.*, London, 1808, pp. 266 and 280; 1809, pp. 415 and 427.

cases irritant effects, probably due to the presence of impurities in the gas, were noticed.

Considerable discussion has arisen concerning the effect of an increased percentage of oxygen in the air breathed upon the respiratory exchange. Is there or is there not an increase in the absorption of oxygen and the discharge of carbon dioxide under these conditions? Many observers [1] maintain that there is a distinct augmentation of the metabolism of the body, others [2] find that the respiratory exchange of a normal animal is the same in amount, whether it breathes air or pure oxygen. Without entering into a discussion of the numerous contradictory answers to this question, it is permissible to draw the following conclusions:—The normal animal does not increase its respiratory exchange when it breathes oxygen instead of air, for its metabolism is regulated by the needs of its tissues, and not directly by the amount of oxygen absorbed in the lungs; in the case of some diseases, during which the blood, owing to diminished absorption of oxygen in the lungs, is abnormally venous, the breathing pure oxygen would increase the percentage of oxygen in the alveolar air, and thus enable the blood in the lungs to take up more oxygen. In these cases breathing oxygen under pressure greater than that of the oxygen in the air would, for a similar reason, be effective, and would also increase the amount of oxygen simply dissolved in the plasma. It would appear, therefore, that there is strictly no contradiction in most of the experimental and clinical results, for in the normal animal breathing ordinary air the arterial blood is almost saturated with oxygen, and without doubt contains as much or more oxygen than the tissues need. This is certainly not the case in some diseases, during which the patients have derived benefit from breathing oxygen.[3]

In connection with the respiration of pure oxygen or of air, Paul Bert [4] made the important discovery that animals exposed to a pressure of oxygen above six atmospheres died in violent convulsions. This result is not due to the purely physical effects of the increased pressure, but to the augmentation in the tension of oxygen, for if the experiment be made with air, a greater and greater pressure can be borne, until

[1] Allen and Pepys, *Phil. Trans.*, London, 1808, pp. 266 and 280; 1809, pp. 415 and 427; Paul Bert, "La pression barométrique," Paris, 1878, p. 832. Further references are given by Phillips, "Materia Medica, Pharmacology, and Therapeutics—Inorganic Substances," London, 1894, 2nd edition, p. 2.

[2] Lavoisier and Sequin, *Hist. Acad. roy. d. sc.*, Paris, 1789, p. 566; Regnault and Reiset, *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi.; Dohmen, "Arb. d. Bonner physiol. Inst.," 1865; Speck, *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xix. S. 171; Kempner, *Arch. f. Physiol.*, Leipzig, 1884, S. 396; Lukjanow. *Ztschr. f. physiol. Chem.*, Strassburg, 1883–84, Bd. viii. S. 313; *Arch. f. Physiol.*, Leipzig, 1884, S. 308. See also references given by Phillips, *loc. cit. supra.*

[3] Ransome, *Med. Chron.*, Manchester, April 1888, May 1889; A. H. Smith, "Oxygen Gas as a Remedy in Disease," New York, 1870, 2nd edition; W. G. Thompson, *Practitioner*, London, 1889, vol. xliii. p. 97. At the end of this article is a list of thirty-two papers on the subject. See also article "Oxygène" in "Dictionnaire de therapeutique, de matière médicale, de pharmacologie, de toxicologie et des eaux minérales," par Dujardin-Beaumetz, Paris, 1889, tome iv. p. 101. See also Phillips, *loc. cit. supra*, and references there given.

[4] Paul Bert, "La pression barométrique," Paris, 1878, p. 800. See also Lehmann, *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiii. S. 173; Liebig, *Arch. f. Physiol.*, Leipzig, 1889, Supp. Bd. S. 41; A. H. Smith, "The Effects of High Atmospheric Pressure, including the Caisson Disease," Brooklyn, 1873; Philippon, *Journ. de l'anat. et physiol. etc.*, Paris, 1894, tome xxx. pp. 296, 414.

a point is reached at which the partial pressure of oxygen becomes dangerous to life. When the arterial blood contains a third more than its normal quantity of oxygen, the metabolism of the body diminishes greatly, and the animal dies. The following examples will illustrate this effect :—

Duration of the Compression.	Pressure in Atmospheres.	Pressure of Oxygen in Atmospheres. $\frac{O \times P}{20{\cdot}9}$	Percentage Composition of Gases of Blood. O_2.	CO_2.	Rectal Temperature.	Remarks.
	Air.	...	14·9	31·1	38°·5	
	$1\frac{3}{4}$	7	21·4	34·3	...	
45 min. .	7	25	32·5	73·8	...	Dog ; convulsions; survived.
	Free air 27 min. after.	...	16·9	21·0	39°·0	
	Free air 67 min. after.	...	17·0	31·5	...	
	Air.	...	19·8	20·9	38°·5	
	Oxygen.	4·4	20·9	34·5	...	
65 min. .	6	24	26·3	63·5	...	Dog ; death.
	9	35	30·7	61·5	...	
	Air.					
	5	21	...	...	33°	Sparrow ; convulsions; death in 30 min.
	Air.					
	8·5	21·5	...	...	...	Sparrow ; convulsions death in 20 min.

The practical importance of these experiments in connection with the symptoms observed in men after working in caissons is obvious. Details of numerous cases are given by Paul Bert [1] and others,[2] but here it is sufficient to draw attention to the chief symptoms and changes observed in men working in compressed air. The earliest and most constant symptom is pain and noise in the ears, due to the pressure upon the tympanum ; relief is generally obtained by swallowing, or by a forced expiration with closed nose and mouth ; in some cases, however, the tympanum has been ruptured. The respiration is slower and deeper. The danger to life, however, chiefly occurs when the workmen leave the caisson and come out into the fresh air ; the symptoms then observed are due to the relative fall in atmospheric pressure, and are chiefly these—very painful itching of the skin, painful swelling of the muscles and joints, disturbances in locomotion and sensation, paralysis of the lower limbs, bladder, and rectum, and more rarely extensive paralysis, unconsciousness, and sudden death.

[1] *Loc. cit.*, p. 369.

[2] See ref. given by Paul Bert, *loc. cit.* ; E. H. Snell, "Compressed Air Illness or so-called Caisson Disease," London, 1896 ; Heller, Mager, Schrötter, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1896, No. 2, S. 40 ; Friedrich and Tauszk, *Wien. klin. Rundschau*, 1896, S. 233.

It is impossible to discuss here the different theories [1] brought forward to explain the symptoms, but it appears that the most probable explanation is that given by Bucquoy,[2] who maintains that the sudden fall in pressure sets free the excess of gases dissolved in the blood during the exposure to the compressed air of the caisson. These particles of gas in some of the small blood vessels would cause embolism, and this would especially affect the nervous system. Great support is given to this theory by the fact that workmen rarely suffer when the change from the compressed air to the open air takes place gradually by a slow fall in pressure, and that the most effective treatment for the more serious symptoms is the subjection of the patient to compressed air. This treatment [3] has been carried out in cases occurring among the workmen employed in the construction of the Blackwall Tunnel under the Thames.

In experiments upon animals, Paul Bert [4] found that the production of carbon dioxide was diminished both when the animal was exposed to a high or to a low atmospheric pressure. Löwy, however, observed no alteration in the respiratory exchange of man, until the pressure of the air fell below 300 mm. There was then an increase in the discharge of carbon dioxide, but no corresponding increase in the intake of oxygen.[5]

A gradual fall in the atmospheric pressure acts upon animals only by decreasing the tension of the oxygen in the air, for, if the percentage of oxygen be raised, a lower pressure can be borne. In air, discomfort is felt when the pressure is reduced to half an atmosphere, and the symptoms become violent with a pressure of 250 mm.; convulsions, insensibility, and death supervene. The limit of pressure appears to be about 200 mm. Such are the results obtained by Paul Bert [6] during experiments upon animals, and they agree with those observed upon man during balloon ascents. Thus during the ascent of the *Zénith* [7] to a height of 8600 metres, Sivel and Crocé-Spinelli died, Tissandier became unconscious, but recovered during the descent; the pressure at that height would correspond to 260 mm., and the tension of oxygen to 52 mm. According to Paul Bert's observations, the oxygen in the arterial blood would be reduced to 10 volumes per cent.

Many theories have been put forward to explain the symptoms of "mountain sickness," but the true one appears to be that of Jourdanet, who maintains that it is due to a condition of anoxyhæmia, a want of sufficient oxygen in the blood.[8] In these cases the absorption of oxygen by the blood would, at the low pressure of the atmosphere, be insufficient for the needs of the tissues of a man or animal engaged in the exertion of climbing. It has been objected [9] that this explanation is incorrect, because there appeared to be no decrease in the amount of oxygen in the blood of dogs, which were subjected by Fränkel and Geppert to a reduced pressure, equal to that of an altitude of

[1] For further details, see Paul Bert's work, *loc. cit.*, p. 520.
[2] "De l'air comprimé," 1861. [3] Snell, *loc. cit.*
[4] *Loc. cit.*, pp. 727, 805.
[5] For observations upon the effect of reduced atmospheric pressure on respiration, see G. v. Liebig, *München. med. Wchnschr.*, 1891, Bd. xxxviii. S. 437; Löwy, *Arch. f. Physiol.*, Leipzig, 1892, S. 545; Speck, *Ztschr. f. klin. Med.*, Berlin, Bd. xii. S. 447.
[6] "La pression barométrique," Paris, 1878, p. 735.
[7] Paul Bert, *loc. cit.*, p. 1061; Tissandier, *Nature*, Paris, 1875, p. 337.
[8] A full discussion will be found in Paul Bert's work, *loc. cit.*, p. 327. See also Clifford Allbutt, "System of Medicine," London, 1897, vol. iii. p. 456. For the effects of high altitudes upon the number of coloured blood corpuscles, see article on "Blood," p. 150.
[9] Gräwitz, *Berl. klin. Wchnschr.*, 1895, S. 713 and 740.

16,000 feet. This may be so during rest, but it is probable that, on exertion, the slower rate of absorption would lead to a deficiency in oxygen; the organism accommodates, not for a condition of rest, but for exertion also; it has a reserve store of energy. Thus, a man with marked anæmia, when he is at rest, absorbs as much oxygen and produces as much carbon dioxide as a healthy man at rest, but, directly marked exertion is necessary, the anæmic subject becomes breathless; he has no reserve upon which to draw during the greatly augmented metabolism which accompanies muscular work.

Nitrogen.—This gas appears to be quite inactive, and an animal confined in it dies from want of oxygen. The same seems to be true for argon. Nitrogen containing 5 per cent. oxygen was found by Sir George Johnson[1] to produce satisfactory anæsthesia in man within a minute; this result is also to be attributed to want of oxygen.

The question of the absorption or discharge of nitrogen by the lungs has been discussed in another part of this work.

Hydrogen.—Numerous experiments have been made upon the effects of respiring hydrogen, and the general conclusion is that it produces no specific effect, but acts only by the exclusion of oxygen.[2] Lavoisier and Seguin found that guinea-pigs respired in a normal manner in a mixture of equal parts of oxygen and hydrogen, and similar results were obtained upon dogs, rabbits, and frogs by Regnault and Reiset. Many cold-blooded animals can live for several hours in pure hydrogen.[3]

Carbon dioxide.—This gas in an undiluted state is irrespirable on account of the spasm of the glottis which it occasions,[4] but when sufficiently diluted with air or oxygen it can be respired, and produces headache, slight giddiness, drowsiness, and hyperpnœa. Some of the earliest experiments with this gas were made by Priestley,[5] who found that cats died from suffocation when placed in carbon dioxide, and butterflies when held over the fermenting liquor in a brewery became motionless in a few minutes, but revived on being brought into the fresh air. Since that time numerous experiments have been made by different observers,[6] especially by Paul Bert, whose results will be mentioned later. Brown-Séquard and d'Arsonval[7] state that they were able to breathe air containing 20 per cent. of pure carbon dioxide for two hours without any marked distress, but it is probable that there was some error in this observation, for Haldane and Lorrain Smith[8] found that when they breathed air containing 18·6 per cent. of this gas the following effects were produced within a minute or two—hyperpnœa, distress, flushing, cyanosis, and mental confusion. Haldane[9] has further investigated this gas in connection with the suffocative gas found in wells and the "black-damp" of mines.

[1] *Lancet*, London, 1891, vol. i.

[2] Scheele, "On Air and Fire," trans. by Forster, London, 1780, p. 160; Fontana, *Phil. Trans.*, London, 1779, vol. lxix. p. 337; *Journ. de phys.*, Paris, tome xv. p. 99; Pilatre de Rozier, *ibid.*, tome xxviii. p. 425; Lavoisier, *Hist. Acad. roy. d. sc.*, Paris, 1789, p. 574; H. Davy, "Researches," p. 465; Allan and Pepys, *Phil. Trans.*, London, 1809, p. 421.

[3] Spallanzani, Edwards, Johannes Müller. See this article, p. 781.

[4] Pilatre de Rozier, *Journ. de phys.*, Paris, tome xxviii. p. 422.

[5] *Phil. Trans.*, London, 1772, vol. lxii. p. 147.

[6] For references, see Benedicenti, *Arch. f. Physiol.*, Leipzig, 1896, S. 408.

[7] *Compt. rend. Acad. d. sc.*, Paris, 1889, 11th Feb.

[8] *Journ. Path. and Bacteriol.*, Edin. and London, 1892, vol. i. p. 175.

[9] *Trans. Fed. Inst. of Mining Engineers*, 1895, vol. viii. p. 549. "The Causes of Death in Colliery Explosions," Government Blue Book, 1896.

Speck [1] found, when he breathed a mixture of gases containing 11·51 per cent. of carbon dioxide, that 528 c.c. carbon dioxide were absorbed by the blood in a minute, whereas under normal conditions 230 c.c. of that gas would have been discharged. In a dog Pflüger [2] found that there were, under normal conditions, 29·8 volumes per cent. carbon dioxide [3] in the arterial blood, but 56·8 volumes per cent. after the dog had breathed for one minute a mixture containing 70 per cent. oxygen and 30 per cent. carbon dioxide. Zuntz [4] observed an increase to 89·6 volumes per cent. carbon dioxide when a dog breathed for one minute and a half a mixture containing 36·9 per cent. carbon dioxide.

Numerous experiments were made by Paul Bert [5] upon the action of this gas upon different forms of life. He found that a percentage of 13·5 to 17 was fatal for reptiles, 24 to 28 for sparrows, and 30 or more for mammals. When the air contained 30 to 40 per cent. of carbon dioxide, death resulted owing to the high tension of the gas in the blood; thus in some dogs the percentage of carbon dioxide in the arterial blood was 116, in the venous blood 120. Complete insensibility could be produced long before any danger to life arose, and thus the gas mixed with air or oxygen could be used for the production of anæsthesia.[6]

Carbon monoxide.—The physiological action of this gas is of the utmost practical importance, since it is every year the cause of numerous deaths in cases of poisoning from coal gas, the fumes of kilns and coke fires, and in the air of coal mines, especially after explosions. Although it has long been known that carbon monoxide is poisonous, it was about the year 1857 that Claude Bernard [7] and Hoppe-Seyler [8] first pointed out that the carbon monoxide displaced the oxygen of the blood by forming a more stable compound with hæmoglobin, and thus brought about asphyxia.[9] The action of this gas has been studied by many observers.[10]

The most recent investigations are those of Haldane,[11] who has experimented both upon himself and upon mice. The following are his chief conclusions:—The symptoms produced in man do not become sensible until sufficient carbonic oxide has been absorbed for the corpuscles to become about a third saturated; with half saturation of the corpuscles the symptoms become urgent. The symptoms are due solely to deficiency in the percentage of oxygen in the blood, and are similar to those experienced by mountaineers and balloonists at high altitudes. The time required for the symptoms to appear in different animals is proportional to the respiratory exchange per unit of body weight, and is about twenty times as long in a man as in a mouse. Hence it is possible with safety to use a mouse as an indicator of the presence of poisonous proportions of carbonic oxide in the atmosphere of a coal-

[1] *Centralbl. f. d. med. Wissensch.*, Berlin, 1876, No. 17.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 103.
[3] Measured at 0° and 1 m.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 408.
[5] "La pression barométrique," Paris, 1878, p. 982. This article, pp. 743–45.
[6] See also Gréhant, *Compt. rend. Soc. de biol.*, Paris, 1887, p. 542.
[7] "Leçons sur les effets des substances toxiques et médicamenteuses," Paris, 1857, p. 184; "Leçons sur les liquides de l'organisme," Paris, 1859, tome i. p. 365; tome ii. p. 427.
[8] *Virchow's Archiv*, Bd. xi. S. 228; Bd. xiii. S. 104.
[9] This Text-book, article "Hæmoglobin."
[10] Gaglio, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 233; Gruber, *Arch. f. Hyg.*, München u. Leipzig, 1883, Bd. i. S. 145; Welitschkowsky, *ibid.*, S. 210; Fokker, *ibid.*, S. 503; Gréhant, "Les poisons de l'air," Paris, 1890.
[11] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 430.

mine. Distinct symptoms are produced by air containing ·05 per cent. of the gas, and urgent symptoms with ·2 per cent. The poisonous action diminishes as the tension of oxygen increases, and *vice versâ*. At a tension of two atmospheres of oxygen this poisonous action is abolished in the case of mice, and this disappearance of the poisonous action is due to the fact that at high tensions of oxygen the animals can dispense entirely with the oxygen-carrying function of hæmoglobin, and can obtain enough oxygen from the gas dissolved in the plasma of the blood.

As regards the gases of the blood, after poisoning with carbon monoxide, Gréhant[1] found that 100 c.c. of blood from the carotid of a poisoned dog contained 6 c.c. of oxygen, 30·3 c.c. of carbon dioxide, and 20 c.c. of carbon monoxide; whereas a sample of blood taken before the administration of the gas yielded 19·5 c.c. of oxygen and 44·2 c.c. of carbon dioxide. The following figures show the effect of different doses of carbon monoxide upon the gases of the blood of dogs poisoned by the gas:—

CO in Inspired Air.	Gases of Blood.			
	CO_2.	O_2.	N.	CO.
1 in 1000 . .	28·9 p. ct.	12·2 p. ct.	1·5 p. ct.	5·5 p. ct.
1 ,, 2000 . .	51·8 ,,	15·5 ,,	1·5 ,,	2·8 ,,
1 ,, 3000 . .	42·2 ,,	13·4 ,,	1·8 ,,	1·7 ,,
1 ,, 4000 . .	40·4 ,,	21·5 ,,	1·5 ,,	1·3 ,,

The administration of small doses of carbon monoxide, enough to produce unconsciousness, causes a marked reduction in the respiratory exchange[2] of a mouse, and its temperature falls.

According to Gaglio,[3] carbon monoxide present in the blood is not oxidised, but St. Martin[4] states that it is slowly oxidised in the presence of oxyhæmoglobin. The compound of this gas with hæmoglobin is partly dissociated in sunlight,[5] but upon these points more details will be given in the discussion upon the gases of the blood.

The respiration of air vitiated by breathing.—The air vitiated by respiration, as in overcrowded rooms, is distinctly unwholesome, but the causes of this deleterious action are not simple, but may arise from substances given off either from the lungs by respiration, from the body by perspiration, or from the injurious products of disease or filth.

Even as early as 1674, Mayow[6] had stated that an animal died if kept in a limited quantity of air, because it had used up the respirable portion, the nitro-aerial gas (oxygen); he further pointed out that respiration and combustion produced similar changes in the air. About the year 1726, Stephen Hales[7] observed by experiments upon himself

[1] *Compt. rend. Soc. de biol.*, Paris, 1892, p. 163.
[2] Haldane, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 430.
[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxii. S. 233.
[4] *Compt. rend. Acad. d. sc.*, Paris, 1891, tome cxii. p. 1232.
[5] Haldane, *loc. cit.*
[6] "Tractatus quinque," Oxonii, 1674.
[7] "Statical Essays," 2nd edition, vol. i. p. 236 *et seq.*

that the "noxious vapours" produced by repeatedly breathing the same air could be removed by potash, and the air rendered fit for respiration. A few years later, Black[1] showed that the "noxious vapours" were carbon dioxide.

The importance of the several factors mentioned above has been differently estimated by various observers.[2] Brown-Séquard and d'Arsonval[3] concluded that volatile poisons were given off from the lungs of healthy men and animals, for they found that the condensed vapour of breath caused death when injected into rabbits; that rabbits made to breathe air vitiated by the respiration of other rabbits until the carbon dioxide was 2 to 6 per cent., died, unless the supposed volatile poisons were removed by previously passing the air over pumice soaked in sulphuric acid; that no bad effects were produced when men breathed for an hour or two air containing 20 per cent. of pure carbon dioxide. The experiment of injecting the condensed vapour of breath has been repeated by Dastre and Loye,[4] Hoffmann-Wellenhof,[5] Lipari and Crisafulli,[6] and Lehmann and Jessen,[7] but the results were negative.

Richardson[8] maintained that breathed air was poisonous, even though all the carbon dioxide and other impurities had been removed; the cause he considered to be "devitalised oxygen," whatever that term may mean. Jackson[9] thought that carbon monoxide was the poison. From experiments performed upon himself, Angus Smith[10] concluded that air vitiated by respiration until it contained 1 per cent. carbon dioxide, produced distinct feelings of discomfort.

Experiments, however, performed by Hermans[11] have shown that no volatile poisons are given off by respiration, and more recently Haldane and Lorrain Smith,[12] in an investigation of the subject, both as regards animals and men, have confirmed and extended Hermans' work. The following are the chief conclusions given by Haldane and Lorrain Smith:—

"1. The immediate dangers from breathing air highly vitiated by respiration arise entirely from the excess of carbon dioxide and deficiency of oxygen, and not from any special poison.

"2. The hyperpnœa is due to excess of carbon dioxide, and is not appreciably affected by the corresponding deficiency of oxygen. The hyperpnœa begins to appear when the carbon dioxide rises to from 3 to 4 per cent. At about 10 per cent. there is extreme distress.

"3. Excess of carbon dioxide is likewise the cause, or at least one cause, of the frontal headache produced by highly vitiated air.

"4. Hyperpnœa from defect of oxygen begins to be appreciable when the oxygen in the air breathed has fallen to a point which seems to

[1] "Lectures on Chemistry," ed. Robison, Edinburgh, 1803.

[2] See Merkel, *Arch. f. Hyg.*, München u. Leipzig, 1892, Bd. xv. S. 1, where further references are given.

[3] *Compt. rend. Acad. d. sc.*, Paris, 1888, tome cvi. pp. 106, 165; *Compt. rend. Soc. de biol.*, Paris, 1887, p. 814; 1888, pp. 33, 90, 99, 151.

[4] *Ibid.*, 1888, pp. 43 and 91.

[5] *Wien. klin. Wchnschr.*, December 13, 1888.

[6] *Bull. gén. de therap.* etc., Paris, 1889, No. 46, p. 524.

[7] *Arch. f. Hyg.*, München u. Leipzig, 1890, Bd. x. S. 367.

[8] *Brit. Med. Journ.*, London, 1860, vol. ii.; *Chem. News*, London, vol. lv. p. 253.

[9] "Proc. Physiol. Soc.," December, 1887, in *Journ. Physiol.*, Cambridge and London, vol. ix.

[10] "Air and Rain," p. 130.

[11] *Arch. f. Hyg.*, München u. Leipzig, 1883, Bd. i.

[12] *Journ. Path. and Bacteriol.*, Edin. and London, 1892, vol. i. p. 175.

differ in different individuals. In one case the hyperpnœa became appreciable at about 12 per cent., and excessive at about 6 per cent."

These observers also point out that the odorous substances arising from want of cleanliness of the body or the room, are also causes of the discomfort experienced in breathing the air of an overcrowded room.

The causes of asphyxia in a limited quantity of air.—A warm-blooded animal confined in a limited quantity of air soon gives signs of discomfort; it becomes restless, breathes more rapidly, and soon pants for breath. This stage is succeeded by one during which the animal is quieter, breathes more slowly but more deeply; it becomes less sensitive, and falls down; agonising efforts are made to breathe, the nostrils are dilated, and the mouth is open. The animal now becomes unconscious, its pupils are dilated, it gives a few slight and irregular respirations, it is seized by convulsions, and then, after a slight pause, its limbs are stretched out with a convulsive shivering movement, its head is thrown back, and it dies.

The general phenomena of asphyxia are described elsewhere in this work;[1] here it is necessary to consider only the chemical changes in the air, the alterations they produce in the respiratory exchange of the animal, and how they cause its death. Upon these questions numerous experiments have been made.[2]

The duration of life in a limited quantity of air depends upon various conditions, such as the amount and temperature of the air, the nature and age of the animal. The following table of some of Paul Bert's experiments will illustrate the influence of some of the above conditions, and will show the composition of the air at the time of death:—

Animal.	Temperature of Air.	Volume of Air.	Duration of Life.	Percentage Composition of Air at the time of Death.	
				O_2.	CO_2.
MAMMALS—					
Cat, 1850 grms. . .	25°	5000 c.c.	25 min.	3·4	17·1
Kitten, 5 days old, 130 grms.	15°	1000 ,,	4½ to 6½ hrs.	2·0	16·6
Kitten, 24 hours old, 125 grms.	11°	435 ,,	1 hr. 15 min.	3·0	14·8
Hedgehog, young, 115 grms.	25°	1500 ,,	1 hr. 15 min.	4·0	14·0
Dormouse, hibernating, 50 grms.	12°	350 ,,	About 1 day	2·2	14·6
Rat, white, 115 grms. .	14°	450 ,,	32 min.	3·0	11·0
,, ,, 125 ,, .	25°	1600 ,,	Between 2 and 3 hrs.	2·2	17·8
,, ,, adult .	30°–35°	2000 ,,	20 min.	11·8	6·5
,, three days old, 5 grms.	25°	100 ,,	More than 6 hrs.	0·75	17·0
Rabbit, young, 200 grms.	25°	6000 ,,	Alive but insensible after 6 hrs.	1·9	13·4
BIRDS—					
Sparrow, 23 grms. .	16°	300 ,,	1 hr.	2·3	13·3
Finch, 25 grms. . .	11°	428 ,,	21 min.	5·0	12·4

[1] Article "Mechanism of Respiration," this Text-book, vol. ii.

[2] Edwards, "De l'influence des agens physiques sur la vie," Paris, 1824; Collard de

Before any conclusions are drawn from the results given in the foregoing table, it will be advisable to consider the cause or causes of death in these cases of asphyxia. Do the animals die from a want of oxygen, or are they poisoned by the accumulation of carbon dioxide? In order to answer this question, experiments have been made on the duration of life of animals confined in air containing an excess of oxygen, or an excess of both oxygen and carbon dioxide. Many observations have been made by various physiologists, but the most complete are those of Paul Bert.[1] The following table gives some of his results:—

An Atmosphere containing an Excess of Oxygen.

Animal.	Temperature of Gases.	Volume of Gases.	Percentage Composition of Gases before the Experiment.		Duration of Life.	Composition of Gases at the time of Death.	
			O_2.	N.		CO_2.	O_2.
WARM-BLOODED—							
Cat, young, 250 grms.	25°	1800 c.c.	55·5	44·5	3 hrs. 25 min.	31	16
Rat, adult, 80 grms.	14°	500 ,,	77	23	1 hr. 45 min.	20	50
Rat, 6 weeks old, 50 grms.	25°	555 ,,	66	34	2 hrs.	29·5	26
Rat, 4 days old.	22°	120 ,,	81	19	18 hrs. 30 min.	28·5	...
Rabbit, young, 200 grms.	22°	1400 ,,	71	29	More than 5 hrs.	43·5	11
Sparrow, young	25°	750 ,,	76	24	More than 5 hrs.	29	...
COLD-BLOODED—							
Grass snake .	...	875 ,,	77	23	8 days	13·5	61
Grey lizard .	27°–29°	570 ,,	79	21	70 hrs.	15·7	...
Toad . .	6°–7°	400 ,,	100	...	7 days	17	81
Frogs . .	6°–7°	400 ,,	100	...	9 days	13·7	8

A consideration of the following results leads to the conclusion, held by Mayow [2] as early as 1674, that a warm-blooded animal confined in a limited quantity of air dies from the want of oxygen, and this conclusion is supported by the fact that its blood is markedly venous and contains little or no oxygen. The percentages of oxygen and of carbon dioxide in the air at the time of death are about 3 and 15 respectively. On the other hand, when there is in the air an abnormal excess of oxygen, and at the same time a great augmentation of carbon dioxide, the warm-blooded animal dies from poisoning with carbon dioxide, and here again the conclusion is strengthened by

Martigny, *Arch. gén. de méd.*, Paris, 1827, tome xiv. p. 203; Snow, *Edin. Med. Journ.*, 1846, vol. lxv. p. 49; Claude Bernard, "Leçons sur les effets des substances toxiques et médicamenteuses," Paris, 1857; W. Müller, *Ann. d. Chem. u. Pharm.*, 1858, Bd. cviii. S. 257; Valentin, *Ztschr. f. rat. Med.*, 1861, Bd. x. S. 33; Beau, *Arch. gén. de méd.*, Paris, 1860, Sér. 5, tome xvi. p. 64; 1864, Sér. 6, tome iii. p. 1; Paul Bert, "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 510.

[1] "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 518.

[2] "Tractatus quinque," Oxonii, 1674. See also this article, p. 741.

the fact that the blood of the animal is generally arterial in colour.[1] The fatal amount of carbon dioxide appears to be about 25 per cent.

An Atmosphere containing an Excess of Oxygen and of Carbon Dioxide.

Animal.	Temperature of Gases.	Volume of Gases.	Percentage Composition of Gases before the Experiment.		Duration of Life.	Composition of Gases at the time of Death.	
			O_2.	CO_2.		CO_2.	O.
WARM-BLOODED—							
Rat, 1 month old, 32 grms.	25°	550 c.c.	90	10	4 hrs.	22·5	77·5
Rat, 1 month old	25°	600 ,,	75	25	20 min.	26·5	73·5
Rat, 3 days old, 5 grms.	25°	150 ,,	80	20	More than 5 hrs.	29·5	...
Mouse, young, 5 grms.	22°	235 ,,	90	10	More than 5 hrs.	24·5	...
COLD-BLOODED—							
Grey lizard .	25°–29	550 ,,	90	10	26 hrs.	16	...
Frog . . .	25°–29°	550 ,,	90	10	20 ,,	17	...

In the cold-blooded animals a marked difference is observed; death in such experiments is generally due to an excess of carbon dioxide, and the fatal percentage, about 16, is much lower than in the case of the warm-blooded animals.

Important differences have also been observed by Edwards[2] and Paul Bert[3] in the duration of life, under water, of animals of different species, and in animals of the same species, but of different ages and exposed to various degrees of external temperature. See table on p. 746.

The importance of these observations lies in the fact that they confirm many of the results obtained by experiments upon the respiratory exchange of different animals. Thus an examination of the above tables shows that the small animals die more quickly than the big animals, and it has been proved that weight for weight they have a more rapid metabolism.[4] Further, a marked difference is observed in hens and ducks, for the latter can live under water three or four times as long as the former. The explanation of this fact is, according to Paul Bert,[5] to be found in the relatively greater quantity of blood in a duck. A similar condition appears to obtain in the seal and whale,[6] which can remain under water from fifteen to thirty minutes.

The tables also show that new-born animals born helpless and blind resist submersion for a much longer time than adults, a fact known and studied by Harvey,[7] Haller,[8] Buffon,[9] and Legallois,[10] but the duration of

[1] Bernard, quoted from Paul Bert, *loc. cit.* p. 522.
[2] "De l'influence des agens physiques sur la vie," Paris, 1824, pp. 629–632.
[3] *Loc. cit.*, p. 534.
[4] This article, p. 720. See also "Animal Heat," this Text-book, vol. i. p. 852.
[5] *Loc. cit.*, p. 550.
[6] Burdach, "Traité de physiologie," trad. par Jourdan, tome vi. p. 122.
[7] "De Generatione," Amst., 1651. [8] "Elementa physiologiæ," 1761, p. 316.
[9] "Histoire naturelle de l'homme."
[10] "Œuvres de Legallois," Paris, 1824, tome i. p. 93.

life under water is much shortened when the temperature of the water is high. The explanation of this is to be sought in the fact that the respiratory exchange of these immature animals is relatively small, and rises and falls with the external temperature.[1]

Animal.	Temperature of Water.	Duration of Life.	Remarks.	Observer.
		Min. Sec.		
MAMMALS—				
Dog	...	4 25	Mean of three experiments.	Paul Bert.
,, five days old .	0°	12 5	,, two ,,	Edwards.
,, ,, .	22·°5	55 30	...	,,
Cat, two months old .	...	2 50	...	Paul Bert.
,, two days old .	0°	4 33	,, nine ,,	Edwards.
,, ,, .	10°	10 23	,, three ,,	,,
,, ,, .	20°	38 45	,, two ,,	,,
,, ,, .	26°	34 30	...	,,
,, ,, .	30°	29 0	,, two ,,	,,
,, ,, .	42°	10 27	,, four ,,	,,
Kitten, very young .	14°	27 30	...	Paul Bert.
,, ,, .	20°	26 0	...	,,
,, ,, .	26°	18 0	...	,,
,, ,, .	36°	11 30	...	,,
Seal, 1 metre long .	...	15–28 0	...	,,
Rabbit . . .	...	3 0	Mean of six experiments; rabbits without food for previous twenty-four hours.	,,
,, ...	...	2 53	Mean of three experiments; rabbits well fed previously.	,,
Guinea-pig . . .	...	2 0	...	,,
,, adult .	...	3 35	Mean of three experiments.	Edwards.
,, two to three days old	...	5 25	,, six ,,	,,
BIRDS—				
Sparrow . . .	0°	0 30	Mean of seven experiments.	,,
,, . . .	20°	0 46	,, ,, ,,	,,
,, . . .	40°	0 39	,, six ,,	,,
,, . . .	...	0 37	,, two ,,	Paul Bert.
Pigeon . . .	...	1 16	,, five ,,	,,
Hen	...	3 31	,, six ,,	,,
Duck	...	11 17	,, eight ,,	,,

The practical importance of these experiments in connection with the cases of suspended animation in children at birth, and in adults after drowning, is obvious.[2]

THE EXCHANGE OF GASES BETWEEN THE BLOOD AND THE AIR IN THE LUNGS—EXTERNAL RESPIRATION.

The mechanism of the ventilation of the lungs is described in another part of this work;[3] here it is necessary only to discuss the frequency and volume of inspiration and expiration, the capacity of the

[1] This article, p. 713. See also "Animal Heat," this Text-book, vol. i. p. 865.
[2] "Report of the Royal Humane Society," 1865, p. 31.
[3] "Mechanism of Respiration," this Text-book, vol. ii.

lungs, and other factors which bear upon the composition of the air in the lungs.

The frequency of respiration in man.—Under normal conditions this could be readily and exactly determined, were it not liable to variations as soon as the attention of the subject is directed to the breathing. Apart from this, the most important causes of variations in the frequency of respiration are age, exercise, and temperature.

Age.—The frequency of breathing decreases from birth to old age, as shown by the following table, the result of three hundred observations made by Quetelet[1] upon human subjects of the male sex.

Age.	Respirations per Minute.		
	Maximum.	Minimum.	Mean.
Newly-born .	70	23	44
5 years .	32	...	26
15–20 ,, .	24	16	20
20–25 ,, .	24	14	18·7
25–30 ,, .	21	15	16·0
30–50 ,, .	23	11	18·1

In healthy infants the respiration is very irregular in frequency, and often of the Cheyne-Stokes type.[2]

The average frequency of respiration in 1897 adult males was found by Hutchinson[3] to be 20 per minute, one-third of the cases breathed at that rate, and 1731 between 16–24 per minute.

Exercise increases not only the frequency but also the depth of breathing. This hyperpnœa is not due to a deficiency of oxygen or an accumulation of carbon dioxide in the blood, but probably to some product which is derived from the metabolism in the muscles, and stimulates the respiratory centre.[4]

The physiological explanation of the condition, well known to athletes as "second wind," appears to be unknown; during violent exercise, such as running or rowing, there is, after a time, considerable dyspnœa, but if the exercise be continued this discomfort disappears, sometimes quite suddenly; the man has now got his "second wind," and can continue the exertion in comparative comfort. The dyspnœa in these cases appears to be partly cardiac, for the pulse-rate may be more than doubled, but when the "second wind" is obtained, there appears to be a marked decrease in the frequency of the heart's contraction.[5] The causes of this accommodation are unknown.

[1] "Sur l'homme et le développement de ses facultés," Paris, 1835.

[2] See Preyer, "Specielle Physiologie des Embryo," Leipzig, 1885, S. 179; Eckerlein, *Ztschr. f. Geburtsh. u. Gynäk.*, Stuttgart, 1890, Bd. xix. S. 120.

[3] *Med.-Chir. Trans.*, London, vol. xxix. p. 137; art. "Thorax," Todd's "Cyclopædia of Anat. and Physiol.," vol. iv. p. 1085.

[4] Geppert and Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 189.

[5] Result of a few observations by Pembrey and Reynolds.

Temperature. — The frequency of respiration is greatly increased when the temperature of the body is raised above the normal by exposure to excessive heat, or by disease; this is especially marked in the dog, for thereby a much greater loss of heat by evaporation of water from the respiratory tract is effected. Richet has shown that this rapid breathing plays an essential part in the regulation of temperature in the dog.[1]

The volume of inspiration and expiration — *Tidal air.* — The earliest determinations of the volume of an ordinary inspiration in man appear to have been made by Borelli,[2] and by Jurin;[3] the latter estimated the amount at 656 c.c., or 40 cubic inches. Since that time numerous determinations have been made with different methods. The following are some of the results:—230 c.c.,[4] 656 c.c.,[5] 574 c.c.,[6 7] 492 c.c.,[8] 278 c.c.,[9] 328 c.c.,[10] 278 c.c.,[11] 197 c.c.,[12] 270 c.c.[13]

The causes of these variations are due to differences in the capacity of the chest of the different subjects of experiment, to individual differences in the breathing, and to imperfections in the methods employed. Vierordt[14] has collected the results of the older observers, and finds as the minimal capacity of a single inspiration 53 c.c. (Abilgaard), as the maximal 792 c.c. (Senebier). From his own numerous determinations Vierordt[15] obtained 446 c.c. as the mean volume of each inspiration, with a frequency of 11·9 per minute, whereas Speck[16] with a frequency of 6·3 respirations per minute found a volume of 1195–1031 c.c. for each inspiration.

Hutchinson[17] has collected the results of different observers, who found for the tidal air volumes varying from 49 to 1640 c.c.; he himself made eighty determinations on different men, and obtained 114–196 c.c. during rest, and 262–360 c.c. during exercise; in one case the tidal air was as high as 1262 c.c.

Marcet[18] found, as the result of 210 experiments upon two men, a mean of 250 c.c. for the tidal air, when the rate of respiration was 16 per minute.

The discrepancy in the results given above is natural; the cases are not comparable as regards the height, weight, age, sex, and development of the different subjects of experiment. It is useless, therefore, to attempt to give any figure which shall represent a true average, and it

[1] See "Animal Heat," this Text-book, vol. i. p. 856; Mathieu and Urbain, *Compt. rend. Acad. d. sc.*, Paris, 1872, tome lxxiv. p. 190.
[2] "De Motu Animalium," p. 2, prop. 81.
[3] *Phil. Trans.*, London, 1717–19, vol. xxx. pp. 757, 758.
[4] Goodwyn, "Connection of Life with Respiration," London, 1788, p. 28.
[5] Menzies, "On Respiration," Edinburgh, 1796, p. 18.
[6] Richerand, "Physiology," trans. by De Lys, p. 206.
[7] Fontana, *Phil. Trans.*, London, 1779, vol. lxix. p. 349.
[8] Dalton, *Mem. Lit. and Phil. Soc. Manchester*, Sér. 2, vol. ii. p. 26.
[9] H. Davy, "Researches," p. 433.
[10] Jurine, "Encyc. Metropol.," art. "Medicine," vol. i. p. 494.
[11] Kite, "Essays," London, 1795, p. 47.
[12] Abernethy, "Essays," 1793, p. 142.
[13] Allen and Pepys, *Phil. Trans.*, London, 1808, p. 256.
[14] Wagner's "Handwörterbuch," Bd. ii. S. 836.
[15] "Physiol. d. Athmens," Karlsruhe, 1845, S. 255.
[16] "Untersuch. ueber Sauerstoffverbrauch u. Kohlensäureausathmung des Menschen," Cassel, 1871, S. 31; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xii. S. 19.
[17] Article "Thorax," Todd's "Cyclopædia of Anatomy and Physiology," vol. iv. p. 1067.
[18] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi.

is much more useful to recognise that the tidal air varies considerably in different individuals, according to the rate and depth of breathing.

The complemental air is the term given to the extra volume of air which can be taken into the lungs by the deepest possible inspiration. Its average value for an adult is said to be 1500 c.c. H. Davy gives 1951 c.c.,[1] Kite 3280,[2] and Hutchinson 1722–1804 c.c.[3]

The reserve or supplemental air is the volume of air which can be expelled after an ordinary expiration by a forcible and deep expiration. This is, according to Bostock's[4] determinations, 2624 c.c. (160 cub. in.), while J. Bell[5] gives 1148 c.c. (70 cub. in.), H. Davy, 1263 c.c. (77 cub. in.), Hutchinson,[6] 1148–1804 c.c. (70–110 cub. in.), and Vierordt, 1226 c.c.

The residual air is the air which remains in the lungs after the most forcible expiration; it cannot be driven out of the lungs during life. The methods[7] employed to determine this volume of air are of two kinds, those for observations on the dead, and those for observations upon the living body. In the first case, the thorax of the corpse is forcibly placed in the position of a deep expiration, and then the air in the lungs is measured. For the determination of the residual air of the living subject, H. Davy[8] introduced an ingenious method; he found by experiment that hydrogen underwent no appreciable change in the lungs, and that it quickly diffused throughout the residual air; he therefore respired a quantity of this gas in a gasometer, and then made a forced expiration, the air of which was analysed. From the quantity of hydrogen left in the lungs, Davy calculated the total quantity of air in the thorax at the end of the forced expiration, and found it to be 672 c.c. (41 cub. in.). This method has been used by Gréhant,[9] and in a modified form by Hermann[10] and Berenstein.[11] Several factors have to be taken into account, such as the absorption of hydrogen by the blood,[12] and its diffusion in the residual air.

Another but less reliable method is Pflüger's[13] pneumonometer. The subject of the experiment, placed in a special chamber, keeps the chest, as far as possible, in the position of a forced expiration, the pressure outside the body is then lowered by a known amount, and the lungs passively give off a certain quantity of air; this volume is measured, and from it and the alteration in pressure the residual air is calculated. The difficulty is to keep the chest in one position during the experiment.

The results obtained by different observers are given in the following table:[14]—

[1] "Chem. and Phil. Remarks," p. 410.
[2] "Essays and Observations, Physical and Medical," 1795, p. 47.
[3] Article "Thorax," Todd's "Cyclopædia of Anatomy and Physiology," vol. iv. p. 1067.
[4] "An Elementary System of Physiology," London, 2nd edition, 1828, vol. ii. p. 25.
[5] "Anatomy," vol. i. p. 193.
[6] "Physiologie des Athmens," Karlsruhe, 1845.
[7] For further details of different methods, see Jacobson, "Beiträge zur Frage nach dem Beitr. der Residualluft," Diss., Königsberg, 1887; and Berenstein, "Ein Beitr. z. Bestimmung der Residualluft," Diss., Dorpat, 1891.
[8] "Researches concerning Nitrous Oxide," London, 1800, p. 399.
[9] *Compt. rend. Acad. d. sc.*, Paris, 1862, tome lv. p. 279; *Journ. de l'anat. et physiol.* etc., Paris, 1864, tome i. p. 523.
[10] "Lehrbuch der Physiol.," Berlin, 1896, Aufl. 11, S. 126.
[11] *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l. S. 363.
[12] Zuntz, Hermann's "Handbuch," Bd. iv., Th. 2, S. 102.
[13] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxix. S. 244.
[14] For some other results, see Hutchinson, *loc. cit.*

Volume of Residual Air in C.c.	Method.	Observer.	Remarks.
1,771	On corpse.	Goodwyn.[1]	Mean of seven experiments.
672·4	On living subject.	H. Davy.[2]	On one subject; hydrogen method used.
1,640	On corpse.	Allen and Pepys.[3]	...
1,230 to 1,640	...	Hutchinson.[4]	...
19,800	On living subject.	Neupauer.[5]	Method defective, results too high.[6]
10,547 to 13,189	,, ,,	Waldenburg.[7]	Method defective, results too high.[6]
1,885	...	Gad.[8]	...
400 to 800	,, ,,	Pflüger.[9]	Pneumonometer used.
500	,, ,,	Kochs.[10]	,, ,,
1,231 max. 640 min. 981 mean	On nine corpses.	Hermann and Jacobson.[11]	...
1,250 max. 440 min. 796 mean	On living subjects, sixteen males.	Hermann and Berenstein.[12]	Hydrogen method used.
526 max. 347 min. 478 mean	On living subjects, three females.	Hermann and Berenstein.[12]	,, ,,

Vital capacity is the term given by Hutchinson to the volume of air which can be expelled from the thorax by the most forcible expiration, following the deepest possible inspiration. The different values assigned to this volume of air are shown in the following table:[13]—

[1] "Connexion of Life with Respiration," London, 1788, p. 25.
[2] "Researches concerning Nitrous Oxide," London, 1800, p. 399.
[3] *Phil. Trans.*, London, 1809, pp. 404, 410, 428.
[4] Article "Thorax," Todd's "Cyclopædia of Anatomy and Physiology," vol. iv. p. 1066.
[5] *Deutsches Arch. f. klin. Med.*, Leipzig, 1879, Bd. xxiii. S. 481.
[6] Zuntz, Hermann's "Handbuch," Bd. iv. Th. 2, S. 103.
[7] *Ztschr. f. klin. Med.*, Berlin, 1879, Bd. i. S. 27.
[8] *Tagebl. d.* 54 *Versamml. deutsch. Naturf. u. Aerzte in Salzburg*, 1881, S. 117.
[9] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxix. S. 244.
[10] *Ztschr. f. klin. Med.*, Berlin, 1884, Bd. vii. S. 487.
[11] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. S. 236, 440.
[12] *Ibid.*, 1891, Bd. l. S. 363.
[13] See also Julius Jeffreys, "Statics of the Human Chest," 1843; Jackson, *Am. Med. Examiner*, 1851, p. 51; Radclyffe Hall, *Trans. Prov. Med. and Surg. Assoc.*, London, 1851.

Vital Capacity.		Observer.	Remarks.
In Cubic Centimetres.	In Cubic Inches.		
3608	220	Jurin.[1]	...
3608	220	Stephen Hales.[2]	...
3493	213	H. Davy.[3]	...
3058	186·5	Thomson.[4]	Mean of twelve experiments.
3280	200	Goodwyn.[5]	...
3280	200	Menzies.[6]	...
4920	300	Kite.[7]	...
3558	217 Mean	Thackrah.[8]	...
4838	295 Max.		
3558	217	Hutchinson.[9]	Mean for 1923 Men.
3700	226	Hermann and Berenstein.[10]	Mean for sixteen Men.

From numerous observations upon men, Hutchinson found that the vital capacity was influenced by the height, weight, and age of the subjects. The following table shows the progression of the vital capacity with the stature[11] :—

Height in Feet and Inches.	Series from Observations on 1012 Cases.	Series from Observations on 1923 Cases.
	Cubic In.	Cubic In.
5·0 / 5·2 } 5·1	175·0	176·0
5·2 / 5·4 } 5·3	188·5	191·0
5·4 / 5·6 } 5·5	206·0	207·0
5·6 / 5·8 } 5·7	222·0	228·0
5·8 / 5·10 } 5·9	237·5	241·0
5·10 / 6·0 } 5·11	254·5	258·0
Mean of all heights	214·0 (3509 c.c.)	217·0 (3558 c.c.)

[1] *Phil. Trans.*, London, vol. xxx. p. 757.
[2] "Statical Essays," 2nd ed., London, 1731, vol. i. p. 243.
[3] "Chem. and Phil. Remarks," p. 410.
[4] "Chemistry of Animal Bodies," 1843, p. 610.
[5] "Connexion of Life with Respiration," London, 1788.
[6] "On Respiration," Edinburgh, 1796.
[7] "Essays and Observations, Physical and Medical," 1795, p. 48.
[8] "On the Effects of Arts, Trades, etc., upon Health," London, 1831, p. 21.
[9] *Loc. cit.* [10] *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l. S. 363.
[11] 1 Foot = 304·8 mm.; 1 inch = 25·4 mm.; 1 cubic inch = 16·4 c.c.

There is an irregular increase of the vital capacity with weight, and as regards age there is an increase from 15 to 35 years, and then a decrease from 35 to 65 years, even when height is taken into consideration. When a man is standing, his vital capacity is 260 cub. in.; in sitting erect, recumbent, and prone positions, it is 255, 230, and 220 cub. in. respectively.

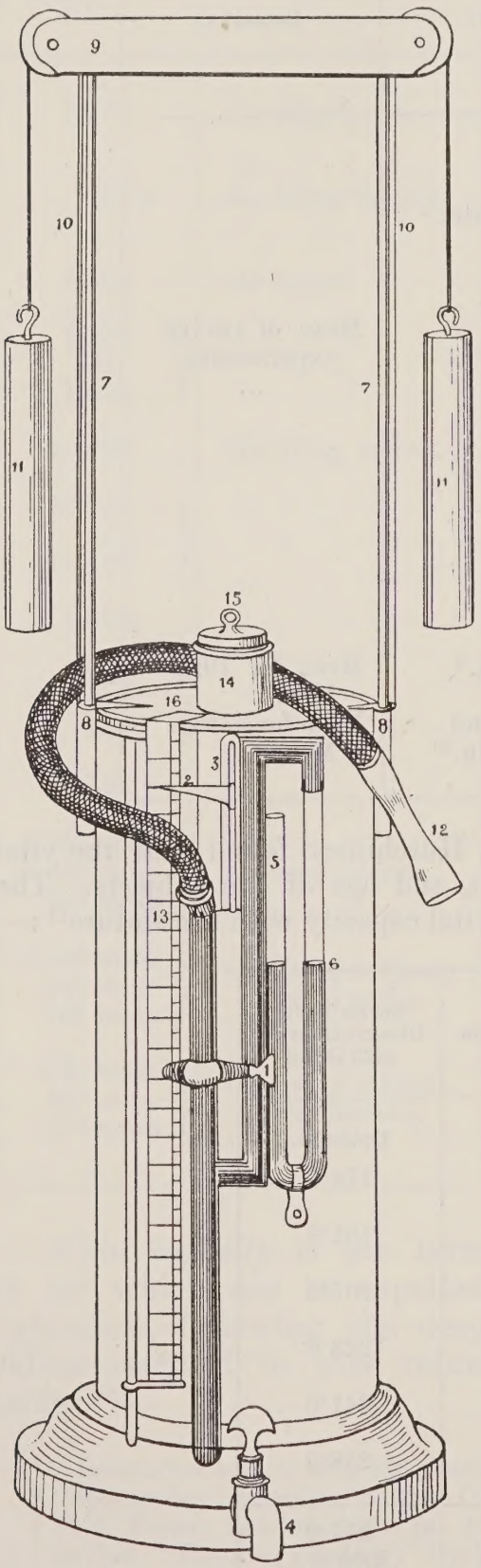


Fig. 68.—Hutchinson's spirometer.

On the opposite page the average amounts of complemental, tidal, reserve, and residual air are given, but it is necessary to point out again that they are only approximate values. The several volumes have already been shown to vary considerably in different individuals.

Hermann[1] subdivides the residual air into *collapse* air, the quantity driven out of the lungs when the thorax is opened; and the *minimal* air, the quantity which remains in the collapsed lungs.

In newly-born children the volume of each inspiration in quiet breathing is 35 c.c., but during screaming it is raised to 61 c.c.; the vital capacity is about 120 c.c. The volume of the lungs of four children born dead at full term was 40, 55, 55, and 60 c.c. respectively, and when blown out they contained 25, 30, 50, and 90 c.c. of air respectively.[2] For the first few days of life the lungs completely fill the opened thorax; there is no collapse air; the residual air is the minimal air. The lungs during each expiration become almost free from air, and the ventilation is very great, the renewal of air being almost perfect.[3]

For the determination of the volumes of air present in the lungs under different conditions, Hutchinson used a special meter, which he termed a *spirometer*. The construction of this apparatus is shown in Fig. 68.[4]

Since that time many simpler and improved forms of spirometer have been introduced.[5] The most important precaution is to reduce the resistance of the meter as much as possible, otherwise the depth and frequency of respiration become abnormal.

[1] "Lehrbuch der Physiol.," Berlin, 1896, Aufl. 11, S. 126.

[2] Eckerlein, *Ztschr. f. Geburtsh. u. Gynäk.*, Stuttgart, 1890, Bd. xix. S. 120.

[3] Hermann, *loc. cit.*, S. 127.

[4] For further details, see Hutchinson, article "Thorax," Todd's "Cyclopædia of Anatomy and Physiology," vol. iv. p. 1069.

[5] Fleischl von Marxow, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1888, S. 39; Clar, *Wien. klin. Wchnschr.*, 1889, No. 18; Marcet, "Proc. Physiol. Soc." *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi.; Hanriot and Richet, *Compt. rend. Soc. de biol.*, Paris, 1887, p. 405.

	Cubic Centimetres.		Cubic Inches.	
Complemental air	1700	Vital capacity, 3500.	104	Vital capacity, 213.
Tidal air	300		18	
Reserve or supplemental air	1500		91	
Residual air	1000		61	

Hyperpnœa, dyspnœa, asphyxia, apnœa, and Cheyne-Stokes' respiration.—These different conditions are considered elsewhere in this work.[1]

The rate of respiration in different animals.—Numerous observations upon the rapidity of the respiratory movements in different animals were made by Paul Bert,[2] and the following table gives results obtained chiefly by him:—

Animal.	Number of Respirations per Minute.	Remarks.	Observer.
MAMMALS—			
Monkey	19	Quiet.	Paul Bert.
Tiger	6	,,	,,
Lion	10	,,	,,
Cat	24	,,	,,
Dog	15	,,	,,
Ox	30	,,	Robertson.[3]
Rabbit	55	,,	Paul Bert.
Rat, black and white	210	,,	,,
Rhinoceros	6	Drowsy.	,,
Horse	10–12	Quiet.	,,
BIRDS—			
Condor	6	,,	,,
Pelican	4	,,	,,
Cock	12	Lying down.	,,
Dove	30	Quiet.	,,
House-sparrow	90	,,	,,
Canary	100	,,	,,
REPTILES—			
Rattle-snake	5	,,	,,
Lizard	12	,,	,,
FISHES—			
Skate (*Raia batis*)	51	,,	Lafont.[4]
Dogfish	40	,,	,,
Perch	30	,,	Paul Bert.
Sole	34	,,	Lafont.
Conger-eel	10	Quiet; length of animal, 1 metre.	Paul Bert.
,,	25	Quiet; length of animal, 50 cm.	,,
CRUSTACEANS—			
King-crab (*Limulus*)	12	Moving.	,,
MOLLUSCS—			
Poulp	28	Quiet.	,,
Cuttlefish	45	,,	Lafont.
Squid	65	,,	Paul Bert.

[1] This article, pp. 743, 765; also "Mechanism of Respiration," this Text-book, vol. ii.

[2] "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 393.

[3] *Veterinary Journal*, London, 1885, vol. xx. p. 311. The rate of respiration in 250 animals varied from 11 to 106 per minute.

[4] Quoted by Paul Bert, *loc. cit.*

The general conclusion to be drawn from these and other similar data is that the larger animals respire more slowly than the smaller animals of a similar class. It has been shown that a similar difference obtains in the output of carbon dioxide and the intake of oxygen.

The alveolar surface of the human lungs.—The volume of the lungs in the mean phase of respiration is about 3500 c.c.; the diameter of a single alveolus is about 0·2 mm., its volume 0·004 c.mm., and its surface 0·126 s.mm. In order to contain the air in the lungs, there must be 725 millions of alveoli, with a surface of about 90 sq. metres.[1] The above calculation is the one given by Zuntz.[2]

The changes in the composition of the air during respiration.—The fresh air taken into the lungs during respiration has the following composition, when it is dry and measured at 0° and 760 mm. pressure, 20·96 volumes per cent. oxygen, 79·02 nitrogen,[3] and about 0·03 carbon dioxide, or by weight per cent., 23·015 oxygen, and 76·985 nitrogen. Under ordinary conditions, the air contains a quantity of aqueous vapour, which is liable to considerable variations according to the temperature and other atmospheric conditions; the carbon dioxide, moreover, may in badly-ventilated rooms rise considerably above the amount just given.

The inspired air is warmed and moistened in passing through the nose, pharynx, trachea, and bronchi, and rapidly mixes and diffuses with the air retained in the alveoli of the lungs. The passage of the air through the nose alone raises the temperature of the air considerably; thus Bloch[4] found that, when the temperature of the external air was –8°, –0°·5 to 3°·5, 12° to 16° and 18°, that of the air entering the pharynx from the nose was respectively 24°·5, 26°, 30°, and 31°. With a moderate external temperature the air becomes about one-third saturated with moisture during its passage through the nasal cavity. The rapidity of the processes of mixture and diffusion will vary according to the frequency and depth of breathing and the capacity of the lungs. The air expired will likewise vary in composition, and under normal conditions will never represent the alveolar air.

The expired air.—The earliest determinations of the composition of the expired air of man were made by Menzies,[5] Lavoisier and Seguin,[6] H. Davy,[7] Allen and Pepys,[8] and Prout.[9] The following table gives the more exact results of recent investigations, but at the same time it is important to remember that it is impossible to give figures which shall exactly represent the average composition of the expired air; the percentage of oxygen and of carbon dioxide varies according to the frequency and depth of breathing, and is influenced by various conditions which affect the metabolism of the body, such as muscular activity, temperature, and food. For these reasons the respiratory exchange of an animal should be estimated by the direct determination of the intake of oxygen and the output of carbon dioxide and water in

[1] 1 mm. = 0·03937 in., and 1 metre = 39·37079 in.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 410.

[3] This includes a small quantity of argon, but it appears to have no physiological importance.

[4] *Ztschr. f. Ohrenh.*, Wiesbaden, Bd. xviii. S. 215, 354.

[5] "Essay on Respiration," Edinburgh, 1796, p. 50.

[6] *Ann. de chim.*, Paris, 1814, tome xci. p. 318.

[7] "Researches," London, 1808, p. 331.

[8] *Phil. Trans.*, London, 1808, 1809.

[9] *Ann. Phil.*, London, 1813, vol. ii. p. 328.

a given time, not by a calculation based upon the alteration in the composition of the air of several expirations, multiplied by the average quantity of expired air and the average number of respirations in a given time.

Breathing.	Volume of Expired Air (per Minute).	Percentage of Oxygen in Expired Air.	Quantity of Oxygen Absorbed (per Minute).	Percentage of Carbon Dioxide in Expired Air.	Quantity of Carbon Dioxide Discharged (per Minute).	Observer.
	c.c.		c.c.		c.c.	
Normal . .	7,527	16·29	358	4·21	318	Speck.[1]
Very shallow .	5,833	15·50	330	4·63	269	
Very deep . .	17,647	18·29	437	3·17	560	
Normal. Rest .	6,158	17·00	240	3·56	218	Speck.[2]
Work . .	16,191	17·29	587	3·65	593	
Hard work . .	24,323	16·96	964	4·08	993	
Normal . .	4,644	16·16	222·9	4·36	202·7	Löwy.[3]
Normal . .	3,419	16·96	136·8	3·44	117·6	

Vierordt[4] concluded from his experiments that the percentage of carbon dioxide in the expired air diminished, but the total discharge increased when the respiration was voluntarily quickened, the depth of breathing remaining the same, 500 c.c.; similar effects were produced by breathing more deeply but with the same frequency. The drawback to these observations is that they were for periods only lasting two or three minutes, and thus they are no exact measure of changes of metabolism. Even the extended observations of Lossen and Berg have been the subject of much discussion and criticism between Pflüger[5] and Voit.[6] It is impossible here to go fully into the causes of some of the contradictory results, but Pflüger appears to have shown that the variations in the breathing have no influence upon the respiratory metabolism beyond this, that when the respiratory muscles are more active, an extra amount of metabolism, due to this activity, will occur. Pflüger takes the mean of the conflicting results and obtains the following suggestive figures:—

Carbon dioxide discharged in fifteen minutes—

Authority.	Five Respirations per Minute.	Sixty Respirations per Minute.
Lossen . .	7·96 grms.	6·63 grms.
Berg . .	7·712 ,,	9·106 ,,
	15·672 ,,	15·736 ,,
	Mean . 7·836 ,,	Mean . 7·868 ,,

[1] *Arch. d. Ver. f. wissensch. Heilk.*, Leipzig, 1867, Bd. iii. S. 317.
[2] "Physiologie des menschlichen Athmens," Leipzig, 1892; *Arch. f. Physiol.*, Leipzig, 1896, S. 465.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xliii. S. 523, *et seq.*
[4] Hesse, *Arch. f. Hyg.*, München u. Leipzig, 1884, Bd. ii. S. 381; "Physiol. d. Athmens," Karlsruhe, 1845, S. 116, 134.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 1, 630.
[6] *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 95.

This conclusion is supported by the work of Pflüger's pupils, Finkler and Oertmann,[1] who found that artificial respiration and apnœa[2] produced no alteration in the absorption of oxygen by rabbits. The respiratory exchange is determined by the activity of the tissues, and not by the frequency of respiration, or the amount of oxygen contained in the blood.

Other changes in the respired air.—It has been shown that in a man at rest the air respired undergoes a reduction in oxygen to about 16 per cent., and an increase in carbon dioxide to about 4 per cent.; in addition, the temperature of the inspired air is raised to that of the body, and this generally occurs before the air reaches the smaller bronchi. At this temperature the air is saturated with moisture, and shows when dried a slight reduction, about $\frac{1}{50}$ in volume, when it is compared with the inspired air, and both are measured at 0° and 760 mm. This decrease in volume is due to the combination and retention of some of the oxygen in the tissues, to the oxidation of some substances which leave the body otherwise than by the lungs, and to the combination of oxygen with hydrogen to form water. The oxygen does not reappear entirely as oxygen in combination with carbon to form carbon dioxide; this is shown by the respiratory quotient, $\frac{CO_2}{O_2}$, which in omnivorous and carnivorous animals is about 0·8. The effect of diet and other conditions upon the respiratory quotient is considered elsewhere in this work, and it has been shown[3] that, under certain conditions, marsh-gas, hydrogen, and nitrogen may be discharged by the lungs.

The Effect of Respiration upon the Blood.

Historical.—The discovery of Harvey that every portion of blood passes through the lungs during each complete circulation, confirmed the idea of the early physiologists, that respiration produced important changes in that fluid; Harvey[4] himself thought that the blood discharged some noxious substances as well as aqueous vapour into the air of the lungs.[5]

In 1669, Lower[6] observed, on opening the thorax of a living animal, and keeping up artificial respiration, that the change of colour from venous to arterial took place in the capillaries of the lungs; the blood in the right ventricle was dark, and if the artificial respiration ceased it passed through the lungs to the left ventricle without attaining an arterial hue; venous blood, when exposed to air outside the body, acquired an arterial colour. Mayow,[7] even earlier than 1674, maintained that this change from venous to arterial colour was due to the absorption by the blood of the nitro-aerial gas (oxygen) from the air in the lungs, but his work was neglected and forgotten.

About the year 1776, Priestley[8] made a series of experiments, in which he showed that dark blood clot became red more rapidly in oxygen than in air, but the red colour was reduced to purple when the clot was placed in nitrogen, hydrogen, or carbon dioxide; these alterations in colour also took place when the blood clot was separated from the air by a piece of moistened bladder, or by a thin film of milk. These changes were supposed by Priestley to be

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 38. See also Pflüger, *ibid.*, S. 9.
[2] See also Hanriot and Richet, *Compt. rend. Acad. d. sc.*, Paris, 1887, tome civ. p. 1327.
[3] This article, pp. 700, 729.
[4] "De Motu Cordis."
[5] For older theories see p. 692, and the references there given.
[6] "Tractatus de Corde," Londini, 1669, pp. 175, 181.
[7] "Tractatus Primus," Oxon., 1674, p. 148.
[8] *Phil. Trans.*, London, 1776, pt. 1, p. 226.

similar to those of combustion, but, biassed by his belief in an old theory, he concluded that the removal of "phlogiston" turned venous into arterial blood, and for this purification respiration was necessary. For many years there were two hypotheses to account for the effect of respiration on the blood. According to the one, which originated apparently with Black,[1] and was accepted by Priestley, Lavoisier,[2] and Crawford,[3] the oxygen in the inspired air combined with the carbon in the venous blood of the lungs, and formed carbon dioxide, which was discharged; whereas, according to the other hypothesis, proposed by Le Grange,[4] the oxygen was absorbed by the blood, and, during the course of the so-called systemic circulation, combined with carbon to form carbon dioxide, which was liberated when the blood again reached the lungs and took up a fresh supply of oxygen.

Notwithstanding the experiments of Spallanzani[5] and of Edwards,[6] which proved that snails, frogs, and kittens continued to give out carbon dioxide in an atmosphere of hydrogen, the view that oxidation took place in the blood was held until recent times, when the work of Pflüger and his pupils showed conclusively that the tissues were the important seat of combustion.

According to Bohr,[7] the tissues of the lungs have a further function than that of simply absorbing and discharging gases; they are said to be able to form carbon dioxide from substances brought to them from other parts of the body. Thus Bohr and Henriques[8] found that the lungs supplied 68 per cent. of the respiratory metabolism. It must be pointed out that in many of the experiments upon which this conclusion is based, the operative procedure was exceedingly severe, and the condition had no approximation to the normal; further, the results are not supported, in fact are contradicted, by the numerous experiments on internal respiration.

The effect of respiration upon the blood is best studied by a comparison of the gases contained in venous blood taken from the right ventricle, and in arterial blood taken from the carotid artery.

The gases of the blood.—Methods for the extraction and estimation of the gases of the blood.—*Historical.*—The first demonstration of the presence of gases in the blood was made by Boyle[9] in 1636; he showed that, when fresh defibrinated blood was exposed to the vacuum of an air-pump, gas was given off. These particles of gas Mayow,[10] in 1674, considered to be nitro-aerial gas, that is, oxygen. The next important observation was that made by Priestley,[11] who noticed that blood placed in an atmosphere of hydrogen or nitrogen gave off oxygen. Girtanner[12] observed the same effect with nitrogen. In 1799, Humphry Davy[13] found that twelve volumes of arterial blood, when heated to 93°, gave off 1·1 volume of carbon dioxide, and 0·7 volume of oxygen.

Nasse,[14] in 1816, proved that blood gave up oxygen to an atmosphere of

[1] "Lectures on Chemistry," edit. by Robison, Edinburgh, 1803.
[2] *Hist. Acad. roy. d. sc.*, Paris, 1777, 1789, 1790.
[3] "On Animal Heat," 2nd edition, 1788.
[4] Hassenfratz, *Ann. de chim.*, Paris, 1791, tome ix. p. 275.
[5] "Mém. sur la respiration," trad. par Senebier, 1803.
[6] "De l'influence des agens physiques sur la vie," Paris, 1824.
[7] *Skandin. Arch. f. Physiol.*, Leipzig, 1891, Bd. ii. S. 236.
[8] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1892, S. 225; *Compt. rend. Acad. d. sc.*, Paris, 1892, tome cxiv. p. 1496.
[9] "Nova experimenta pneumatica respirationem spectantia," Genevæ, 1636.
[10] "Tractatus quinque," Oxonii, 1674. "Opera omnia," Hagae Com., 1681, p. 133.
[11] *Phil. Trans.*, London, 1776, pt. 1, p. 226.
[12] Hassenfratz, *Ann. de chim.*, Paris, 1791, tome ix. p. 275.
[13] *Ann. d. Phys. u. Chem.*, Leipzig, 1803, Bd. xii. S. 574, 593.
[14] *Deutsches Arch. f. d. Physiol.*, Halle, 1816, Bd. ii. S. 195, 435.

hydrogen, or of carbon dioxide, and Vogel,[1] in 1814, and Collard de Martigny,[2] in 1830, obtained carbon dioxide, but no oxygen, from blood subjected to a vacuum. Notwithstanding these observations, the presence of gases in the blood was for a long time a subject of controversy. Many physiologists, among them Johannes Müller,[3] Schroeder van der Kolk,[4] Gmelin,[5] Mitscherlich,[5] and Tiedemann,[5] maintained that no gas existed in the blood, whereas Nasse,[6] Scudamore,[7] Bischoff,[8] and Van Euschut[9] obtained from blood carbon dioxide, but no oxygen. John Davy was at first[10] unable to extract any gas from blood, but during a further research he obtained carbon dioxide from both arterial and venous blood.[11]

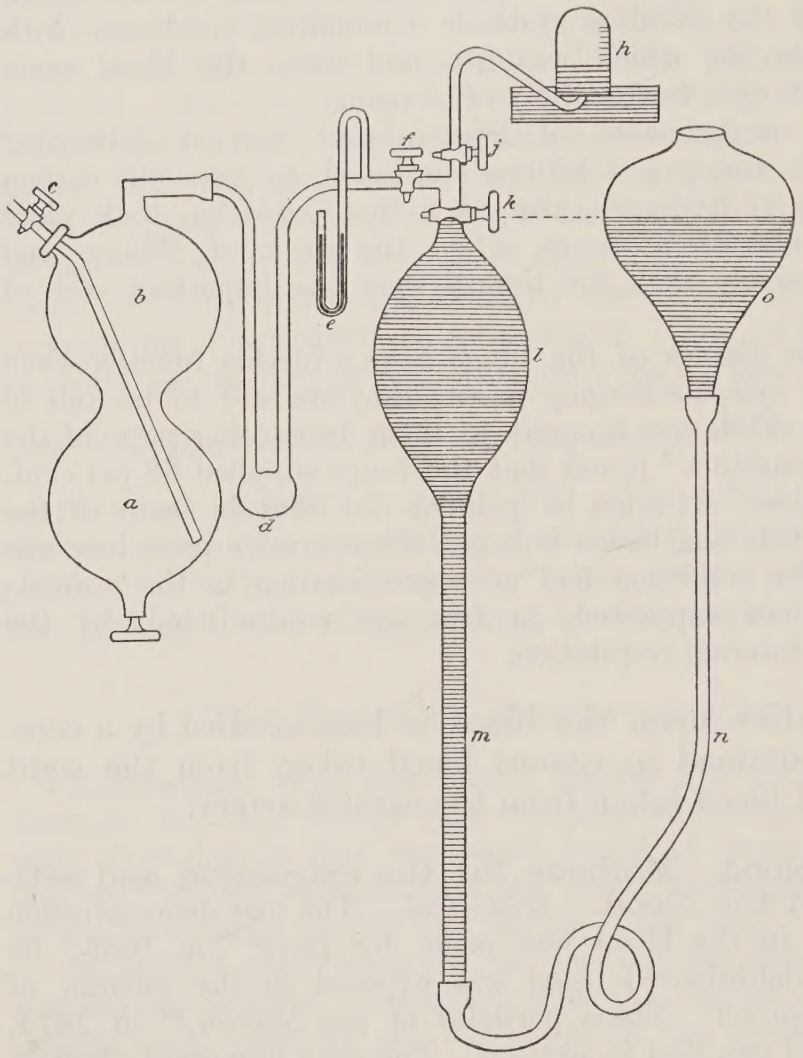


FIG. 69.—Pflüger's Pump; *a*, blood bulb; *b*, froth-chamber; *d*, drying tube; *e*, mercurial gauge; *h*, graduated tube for collection of gas; *l*, *m*, *n*, and *o*, bulbs and tubing containing mercury.

More exact methods of observation were introduced in 1837 by Magnus,[12] who adopted and improved, for the extraction of the gases, the use of a Torricellian vacuum, a method due originally to Collard de Martigny. The conclusions to which Magnus arrived were that blood contained 4–8 volumes per cent. carbon dioxide, 1–3·5 volumes per cent. oxygen, and 0·5 – 2 volumes per cent. nitrogen, and that arterial blood contained more oxygen than did venous blood. Fernet,[13] in 1857, published the results of experiments in which he had extracted the gases of the blood by the passage of a stream of hydrogen, and the aid of a vacuum. About the same time, Lothar Meyer[14] developed the method[15] of heating the blood or other liquid for the extraction of its gases, and a still further advance was

[1] *Journ. f. Chem. u. Phys.*, Nürnberg, 1814, Bd. xi. S. 399.
[2] *Journ. de physiol. expér.*, Paris, 1830, tome x. p. 111.
[3] "Handbuch d. Physiol.," Bd. i. S. 315.
[4] "Dissertatio sistens sanguinis coagulantis historiam."
[5] *Ztschr. f. Physiol.*, 1833, Bd. v. S. 6.
[6] *Loc. cit.*
[7] "An Essay on the Blood," London, 1824.
[8] "Commentatio, etc.," Heidelbergæ, 1837.
[9] "De respirationis Chymismo," Trajecti ad Rhenum, 1836, pp. 78, 84, 98, 115, 142.
[10] *Phil. Trans.*, London, 1823, p. 516.
[11] "Researches," London, 1839, vol. ii. p. 156, *et seq.*
[12] *Ann. d. Phys. u. Chem.*, Leipzig, 1837, Bd. xl. S. 583; 1845, Bd. lxvi. S. 177.
[13] *Ann. d. sc. nat.*, Paris, 1857, Sér. 4, Zool., tome viii. p. 125.
[14] "Die Gase des Blutes," Göttingen, 1857: *Ztschr. f. rat. Med.*, N.F., Bd. viii. S. 256.
[15] Used originally by H. Davy, Bunsen, and Baumert.

made when Ludwig and Setschenow,[1] Pflüger[2] and Helmholtz,[3] constructed their mercurial gas-pumps, based upon the principle of the Torricellian vacuum.

The mercurial gas-pump.—Numerous forms[4] of this apparatus have been introduced, but here it is only necessary to mention Pflüger's pump, the modification of this made by Gréhant,[5] and the simple apparatus devised by Leonard Hill. The principle of the first is shown in the diagram on p. 758.

Further details upon the construction and working of these pumps will be found in text-books of physiological chemistry.[6]

In Leonard Hill's[7] gas-pump, the chief advantages are simplicity, cheapness, and rapidity of action; the working errors are under 1 per cent., and only small quantities of blood are required. The construction of the pump is shown in Fig. 70, and the successive manipulations are as follows:—"A blood-receiver (F) is affixed to the end of the tube E, and the receiver is elevated into the position indicated by the dotted outline. The reservoir (B) is then put in connection with the tube (E) by means of the three-way tap (D), the reservoir (A) is raised above the pump, and the whole system is filled with mercury to the top of the blood-receiver (F). The screw-clip on the rubber tube at the upper end of F is then closed, and the reservoir (A) lowered until the blood-receiver is exhausted, except for 2 or 3 c.c. of mercury, which is purposely left within. The screw-clip on the lower end of F is next closed, and the blood-receiver now clipped at either end, exhausted, detached from tube E, and weighed. A sample of blood is then collected. The arterial or venous cannula is filled with blood, and immediately afterwards pushed into the rubber tube at the

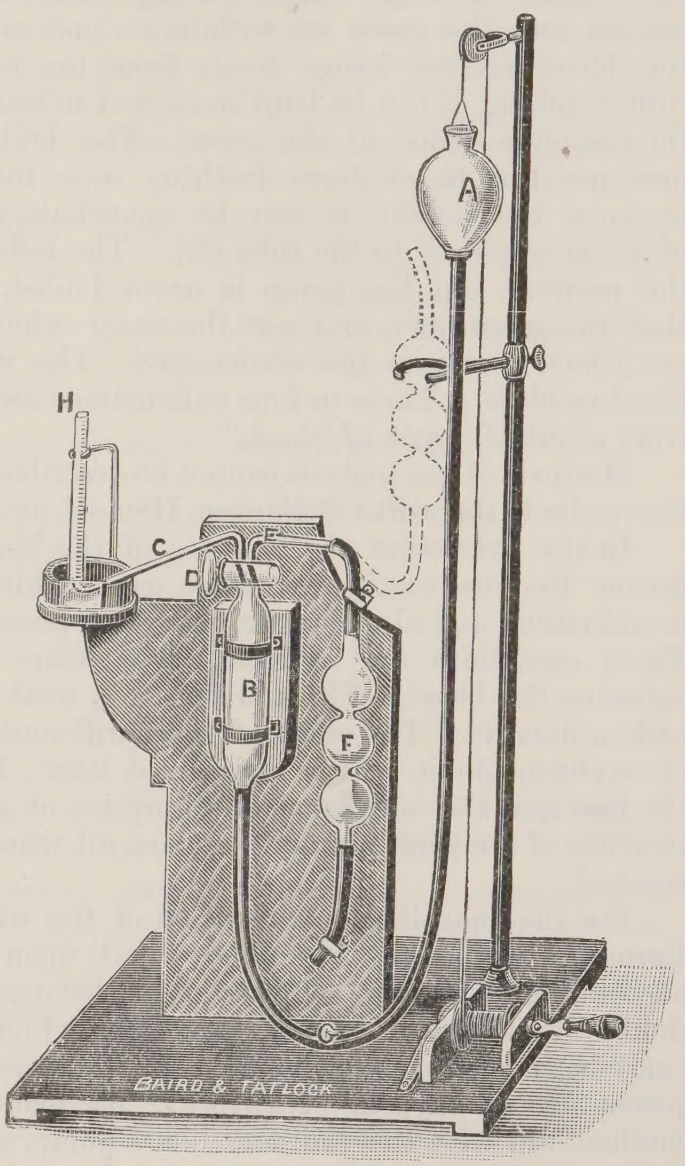


FIG. 70.—Leonard Hill's Gas-Pump.

[1] *Sitzungsb. d. k. Akad. d. Wissensch. Math-phys. Cl.*, Wien, 1859, Bd. xxxvi. S. 293.

[2] "Untersuch. a. d. Bonner physiol. Lab.," 1865, S. 188; *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, S. 305; *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 61.

[3] See Zuntz, Hermann's "Handbuch," Bd. iv. Th. 2, S. 27.

[4] A. Schmidt, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math-phys. Cl.*, Leipzig, 1867, Bd. xix. S. 33; Hoppe-Seyler, "Physiol. Chem.," Berlin, 1879, Bd. iii. S. 491; Nawrocki, *Stud. d. physiol. Inst. zu Breslau*, Leipzig, Bd. ii. S. 144; Busch, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 445; Kossel and Raps, *Arch. f. Physiol.*, Leipzig, 1893, S. 198.

[5] Paul Bert, "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 102; "La pression barométrique," Paris, 1878, p. 615.

[6] Halliburton, "Text-Book of Chemical Physiology and Pathology," London, 1891, p. 30; Hempel, "Gasanalytische Methoden;" Gamgee, "Physiological Chemistry of the Animal Body," vol. i. pp. 200–206.

[7] *Journ. Physiol.*, Cambridge and London, 1894-5, vol. xvii. p. 353; Hill and Nabarro, *ibid.*, 1895, vol. xviii. p. 218.

end of the blood-receiver, as far as the closed screw-clip. Before the insertion of the cannula, the end of the rubber tube is compressed with the fingers to exclude the air within it. A sufficient quantity of blood is now withdrawn by opening at the same time the screw-clip and the clip placed on the blood vessel of the animal. The blood is defibrinated by shaking it with the mercury left within the blood-receiver for that purpose, and the latter is then again weighed. The weight of the sample of blood is then obtained. The blood-receiver is next affixed once more to the tube (E), in the dependent position shown in the figure, and the tube (E) is exhausted. Finally, the screw-clip between E and the blood-receiver is opened, and the gases are withdrawn and collected in the eudiometer. Since the blood-receiver hangs freely from the tube (E) by means of a piece of rubber tubing, it can be both immersed in warm water, and shaken to facilitate the complete escape of the gases. The bulbous form of the blood-receiver prevents the blood from frothing over into the pump; and if the action becomes too violent, it can be immediately allayed by pouring a few drops of warm water on to the tube (E). The bubbles are thereby driven back into the receiver, and the pump is never fouled. The tap (D) is so manipulated that the gases only, and not the water which condenses in the reservoir (B), are driven over into the eudiometer. The water is returned back into the blood-receiver. Three or four exhaustions are sufficient to extract all the gases from about 10 grms. of blood."

Methods of gas analysis cannot be described here; it is only necessary to refer the reader to the works of Bunsen, Hempel, and others[1] upon this special subject.

In the extraction of the gases of the blood methods are employed which favour the dissociation of those gases which are present in loose chemical combination, and also liberate the gases present in a state of simple solution. These conditions are fulfilled by exposure to a vacuum, by warming and agitating the blood. The addition of a weak acid favours the evolution of the carbon dioxide. The effect of these different procedures upon the dissociation of oxyhæmoglobin will be considered later; here it is only necessary to recall the fact that the coefficient of absorption of gases in fluids diminishes with an increase of temperature, and becomes nil when the boiling point of the fluid is reached.

For the quantitative estimation of the oxygen contained in blood, Claude Bernard[2] introduced a method based upon the stronger affinity shown by carbon monoxide than by oxygen for hæmoglobin. The blood is shaken with double its volume of carbon monoxide, which drives out the oxygen from its combination with hæmoglobin. An analysis of the gas collected shows the percentage of oxygen. Nawrocki[3] has made comparative analyses with this method and with the ordinary blood pump, and the results are practically the same. If, however, the blood is left in contact with the carbon monoxide for longer than twenty-four hours, some of the gas combines with oxygen to form carbon dioxide, and thus the amount of oxygen is diminished.[4] It is possible that the carbon dioxide formed in these cases is due to putrefaction.

The differences in the gases of arterial and venous blood.—A comparative examination of the gases contained in arterial and venous blood is necessary for the estimation of the qualitative and quantitative changes which occur during external and internal respiration.

The gases of arterial blood.—The chief results obtained by

[1] Bunsen, "Gasometrische Methoden," 1857; "Gasometry," Roscoe's transl., London, 1857; cf. also Gamgee, *op. cit.*, pp. 206-215; Hempel, "Gasanalytische Methoden"; Geppert, "Die Gasanalyse," 1885.

[2] "Leçons sur les liquides de l'organisme," Paris, 1859, tome i. p. 365; ii. p. 427.

[3] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, Bd. ii. S. 144.

[4] Bernard, *loc. cit.*, Pokrowsky, *Virchow's Archiv*, 1866, Bd. xxxvi. S. 482.

different observers upon various animals have been collected in tabular form by Zuntz,[1] and are here reproduced with some additions:—

Animal.		Quantity of Gas in Percentage of Volume of Blood, Measured at 0° and 760 Mm.			Observer and Number of Experiments.
		Oxygen.	Nitrogen.	Carbon Dioxide.	
Dog . .	Mean	22·6	1·8	34·3	Pflüger,[2] twelve experiments, but only three determinations of CO_2. Rapid method employed.
Dog . .	Mean	18·3	1·8	37·7	Forty-four analyses of carotid blood by Setschenow, Schœffer, Sczelkow, Nawrocki, Hirschmann, Sachs and Pflüger. Collected by Pflüger.[3]
	Max.	24·7	5·5	53·4	
	Min.	11·4	1·2	23·3	
Dog . .	Mean	18·4	2·0	38·8	Blood of femoral artery. Twenty-five analyses by Pflüger,[3] two by Hirschmann.
Dog . .	Mean	19·4	...	40·4	One hundred experiments by Paul Bert.[4]
	Max.	26·4	...	50·8	
	Min.	14·4	...	33·0	
Dog . .	...	17·58	...	38·57	Geppert and Zuntz.[5]
Dog . .	...	15·4	1·5	40·1	Gréhant.[6]
	...	21·2	1·5	45·9	
	...	15·2	1·8	40·0	
	...	22·7	1·5	40·4	
Dog . .	...	17·3	...	33·4	Ewa ld.[7]
	...	15·4	...	32·4	
	...	15·8	...	35·1	
	...	15·7	...	26·35	
Dog . .	Mean	18·25	...	37·64	Hill and Nabarro,[8] average of fifty-two samples of arterial blood.
Cat . .	Mean	13·1	1·3	28·8	P. Hering,[9] six experiments. The value of oxygen is too low, since phosphoric acid was added to the blood beforehand.
Sheep . .	Mean	10·7	1·8	45·1	Sczelkow.[10] Two analyses.
Sheep . .	Mean	12·8	...	...	Three analyses by Preyer[11] on venous blood shaken with air.
Rabbit .	Mean	13·2	2·1	34·0	Four analyses by Walter.[12]
Rabbit .	...	10·9	...	37·34	Geppert and Zuntz.[13]
Man . .	...	21·6	1·5	40·3	One analysis by Setschenow.

[1] Article in Hermann's "Handbuch," Bd. iv. Th. 2, S. 35.
[2] *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 722.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 274.
[4] "La pression barométrique," Paris, 1878, p. 1030.
[5] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xlii. S. 189.
[6] *Compt. rend. Soc. de biol.*, Paris, 1892, p. 163.
[7] *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii. S. 575.
[8] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. pp. 223, 224, 227.
[9] "Untersuch. u. d. Zusammensetzung der Blutgase während der Apnoe," Diss., Dorpat, 1867, *Meissner's Jahresb.*, 1867, S. 305.
[10] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1864, S. 516.
[11] *Wien. med. Jahrb.*, 1865, S. 145.
[12] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. vii. S. 148.
[13] *Arch. f. d. ges. Physiol.*, Bonn, Bd. xlii. S. 189.

The results of analyses of the gases in the blood of birds and of other animals are given by Zuntz.[1]

Estor and Saint-Pierre[2] concluded, from a few analyses of arterial blood, that the amount of oxygen diminished in proportion to the distance of the artery from the heart; these results, however, have been shown by Paul Bert,[3] Hirschmann,[4] and Pflüger[5] to be erroneous. The blood in the smaller arteries contains less oxygen, but this is independent of the distance from the heart, and appears to be due to the smaller number of red corpuscles, and the lower specific gravity of the blood.[6]

The results given in the above table show considerable differences in the percentage composition of the gases of arterial blood, even when the experiments have been made upon similar animals. The causes of these differences are partly due to variations in the gases in the blood, and partly to errors of analysis. In order to test these points, double analyses of portions of the same blood have been made by Preyer and Ludwig,[7] Pflüger,[8] and others.[9] The most important discovery in this connection is that made by Pflüger;[10] the ordinary methods for the extraction of the gases of the blood give results which for the oxygen are too low, for the carbon dioxide too high; arterial blood, when removed from the body, and kept from contact with the air, rapidly becomes darker. Some of the oxygen appears to be used up by the corpuscles with the production of carbon dioxide. If the blood be received directly from the artery into a large vacuum, and the gases quickly extracted, then values are obtained which show a higher percentage of oxygen, and a lower percentage of carbon dioxide than those found by the ordinary slower methods. The normal amount of oxygen in the fresh arterial blood of the dog is about 22 per cent. The carbon dioxide naturally shows considerable variations, but the amount of nitrogen in the most exact determinations is fairly constant, about 1·8 per cent.

The arterial blood is not quite saturated with oxygen, for by rapid artificial respiration in the living animal, or by shaking arterial blood with air, the amount can be raised above 23 volumes per cent.[11] Geppert and Zuntz found in the arterial blood of dogs a relative saturation with oxygen of 96–99 per cent.[12] The quantity of carbon dioxide in arterial blood is only about one-fifth of the amount which can be held by the blood, for Paul Bert[13] found that dog's blood could take up about 150 volumes per cent. when shaken with pure carbon dioxide. The nitrogen is simply in solution and the blood appears to be saturated with that gas, for the ordinary pressure and temperature.

The gases of venous blood.—On account of the differences in the metabolism of the different tissues of the body, the venous blood is liable

[1] Article in Hermann's "Handbuch," Bd. iv. Th. 2, S. 41.
[2] *Journ. de l'anat. et physiol. etc.*, Paris, 1865, tome ii. p. 302.
[3] "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 118.
[4] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1866, S. 502.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 274.
[6] Mathieu and Urbain, *Arch. de physiol. norm. et path.*, Paris, 1871, tome iv.; Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 75.
[7] *Wien. med. Jahrb.*, 1865, Bd. xxi. S. 145.
[8] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 61.
[9] Hammarsten, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Cl.*, Leipzig, 1871, Bd. xxiii. S. 630; Afanassiew, *ibid.*, 1872, Bd. xxiv. S. 256; Tschiriew, *ibid.*, 1874, Bd. xxvi. S. 120.
[10] *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 321, 722.
[11] Setschenow, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. xxxvi. S. 289; Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 70; Ewald, *ibid.*, 1873, Bd. vii. S. 575.
[12] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 242.
[13] "La pression barométrique," Paris, 1878, p. 1038.

to corresponding variations in its gaseous constituents. It is therefore only possible to give a mean value for the gases of the venous blood when the analyses are performed upon samples removed from the right ventricle; this procedure can be carried out by passing a catheter from the right external jugular vein through the right auricle and into the right ventricle. The following table gives the results of the few experiments of this kind, together with the data of simultaneous analyses of the arterial blood:—

Animal.	Venous Blood from Right Ventricle.			Arterial Blood.			Observer.
	Oxygen.	Nitrogen.	Carbon dioxide.	Oxygen.	Nitrogen.	Carbon dioxide.	
Dog . .	11·9	1·7	45·3	19·2	2·7	39·5	Schœffer [1] (mean of five experiments).
,, . .	5·5	...	56·4	22·1	...	36·1	Paul Bert.[2]
,, . .	...	...	49·0	19·3	...	38·7	
,, . .	11·7	...	36·5	17·3	...	33·4	Ewald.[3]
,, . .	9·5	...	34·0	15·4	...	32·4	
,, . .	12·5	...	36·0	16·5	...	34·8	Finkler.[4]
,, . .	12·5	...	24·96	16·12	...	30·65	
,, . .	9·9	...	54·75	17·25	...	42·75	Mathieu and Urbain.[5]
,, . .	5·43	...	61·08	20·75	...	47·33	

The effect of different conditions on the gases of venous blood.—It has already been mentioned that the venous blood is liable to marked differences in its gaseous contents, according to the condition of the organs from which the blood is received.

The venous blood leaving a muscle varies according to the condition of the tissues[6]; when the muscle is actively contracting, the percentage of oxygen in the blood is much diminished, and the amount of carbon dioxide is increased, notwithstanding the increase in the volume and velocity of the circulating blood. The experiments of Bernard[7] and Zuntz[8] show that, after section of the motor nerve, the absorption of oxygen and the production of carbon dioxide in the muscle are much

[1] *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1860, Bd. xli. S. 589.

[2] "La pression barométrique," Paris, 1878, p. 1038.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii. S. 575.

[4] *Ibid.*, 1875, Bd. x. S. 368.

[5] *Compt. rend. Acad. d. sc.*, Paris, 1872, tome lxxiv. p. 190.

[6] Sczelkow, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlv. S. 171; Mathieu and Urbain, *Compt. rend. Acad. d. sc.*, Paris, 1872, tome lxxiv. p. 190; Bernard, "Leçons sur la chaleur animale," Paris, 1876, p. 147; Zuntz, *Berl. klin. Wchnschr.*, 1878, No. 10, S. 141; von Frey, *Arch. f. Physiol.*, Leipzig, 1885, S. 533; Chauveau and Kaufmann, *Compt. rend. Acad. d. sc.*, Paris, 1886, tome ciii. pp. 974, 1057, 1153; Hill and Nabarro, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 218.

[7] *Loc. cit.*

[8] *Loc. cit.*

less than in the normal condition, the blood undergoing comparatively slight changes in its passage through the capillaries.

The following are some of the results obtained by Zuntz:—

Blood Vessel.	Percentage Volume of Gases in Blood.		Remarks.
	Oxygen.	Carbon dioxide.	
Femoral vein . . .	1·2	36·32	Dog at rest.
Carotid artery . . .	14·4	21·92	
In the muscles of right limb were absorbed and produced respectively .	13·20	14·40	...
Femoral vein . . .	2·85	33·16	After section of sciatic and crural nerves on the right side.
Carotid artery . . .	13·30	23·06	
In the muscles of right limb were absorbed and produced respectively .	10·45	10·1	...

Calculated from these results, the respiratory exchange before the section of the nerves was 1·21 c.c. oxygen and 1·32 c.c. carbon dioxide per minute; after section of the nerves, 0·68 c.c. oxygen and 0·65 c.c. carbon dioxide.

Chauveau and Kaufmann[1] estimated the gaseous exchange in the masticatory muscles of the horse, both when it was at rest, and when it was actively chewing. The following table gives their results, together with those of somewhat similar experiments made by Sczelkow,[2] Hill and Nabarro:[3]—

	Difference between the Venous and Arterial Blood.			Observer.
	Rest.	Activity.		
Carbon dioxide	+6·71	+10·79 × 3 = +32·37		Sczelkow.
Oxygen . .	−9	−12·26 × 3 = −36·78		
Carbon dioxide	+8·7	+10·20 × 3 = +30·60		Chauveau and Kaufmann.
Oxygen . .	−11·4	−13·65 × 3 = −40·95		
		Tonic	Clonic.	
Carbon dioxide	+8·76	+13·90 × 3 = +41·70	+19·33 × 3 = +57·99	Hill and Nabarro.
Oxygen . .	−12·92	−13·75 × 3 = −41·25	−12·63 × 3 = −37·89	

In the above table the amounts found during activity are multiplied

[1] *Compt. rend. Acad. d. sc.*, Paris, 1886, tome ciii. pp. 974, 1057, 1153.
[2] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlv. S. 171.
[3] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 218.

by three, in order that allowance may be made for the increased rate of flow in the blood of an active limb.

As regards the velocity of the circulation, Finkler[1] finds that the difference between the arterial and venous blood increases as the velocity diminishes. This relationship is well shown by Bernard's[2] observations upon the submaxillary gland. When the gland is at rest the venous blood is dark, but becomes almost arterial in colour when the gland becomes active and its blood vessels are dilated by stimulation of the chorda tympani. The difference between the arterial and venous blood is less marked, but the total absorption of oxygen and production of carbon dioxide are increased.

In the last stage of asphyxia, the arterial blood contains only traces of oxygen. Thus Ludwig[3] gives, as the result of six analyses made by Setschenow and Holmgren upon asphyxiated dogs, 0·4 volume per cent. oxygen, 3 per cent. nitrogen, and 54 per cent. carbon dioxide; and Zuntz[4] has collected the results of nineteen analyses made by different observers,[5] and obtains an average of 0·96 volume per cent. oxygen, 2·07 per cent. nitrogen, 49·53 per cent. carbon dioxide. These values Zuntz contrasts with those obtained from averages of seventy-one analyses made by Pflüger and others upon normal arterial blood, namely, 18·3 volumes per cent. oxygen, 1·9 per cent. nitrogen, and 38·1 carbon dioxide; and he shows that the ratio between the increase of carbon dioxide and the loss of oxygen is 0·66 in asphyxia, as compared with 0·79 in the normal condition. This difference is to be explained by the retention of some carbon dioxide in the tissues, owing to the high tension of that gas in the blood.

During apnœa the arterial blood is almost saturated with oxygen, and contains about one-half its normal amount of carbon dioxide; the venous blood, on the other hand, contains less oxygen as well as less carbon dioxide than it does in the normal condition.[6] These results confirm the work of Pflüger,[7] who found that during apnœa the respiratory exchange was not greater or smaller than in the ordinary condition of respiration.

The changes which the blood undergoes in passing through the brain are much less marked than those which occur during its passage through muscles. Even during marked activity the brain has a comparatively small respiratory exchange.[8]

The nature of the connection between the blood and its gases.—Oxygen.—Magnus[9] in 1836 concluded that the gases of the blood were simply dissolved in that fluid, notwithstanding the fact that his experiments showed that the quantity of oxygen in the blood was much greater than the amount which could be dissolved in an equal volume of water exposed to air. Justus Liebig,[10] however, pointed out that Regnault and Reiset's[11] experiments showed that animals absorbed the same amount of oxygen whether they breathed pure oxygen or air; he therefore urged

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. x. S. 368.

[2] "Leçons sur les liquides de l'organisme," Paris, 1859, tome ii. p. 435; "Leçons sur la chaleur animale," Paris, 1876, p. 185.

[3] *Wien. med. Jahrb.*, 1865, Bd. xxi. S. 145.

[4] Hermann's "Handbuch," Bd. iv. Th. 2, S. 43.

[5] See Zuntz, *loc. cit.*

[6] Ewald, *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii. S. 575.

[7] *Ibid.*, 1868, Bd. i. S. 100.

[8] Hill and Nabarro, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 218. See also "Animal Heat," this Text-book, vol. i. p. 808.

[9] *Ann. d. Phys. u. Chem.*, Leipzig, 1837, Bd. xl. S. 583; 1845, Bd. lxvi. S. 177.

[10] *Ann. d. Chem. u. Pharm.*, 1851, Bd. lxxix. S. 112.

[11] *Ann. de chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi.

that the gases of the blood were present in a state of loose chemical combination with some unknown constituent of the blood, in a similar way to that in which carbon dioxide is combined in solutions of sodium phosphate. A few years later, Lothar Meyer [1] came to a similar conclusion, for he found that the amount of oxygen retained in the blood only varied slightly with alterations of pressure. About the same time Fernet [2] observed that the amount of oxygen chemically combined in blood saturated with air was about five times greater than the quantity which could be dissolved at the ordinary atmospheric pressure; this oxygen was, moreover, chiefly contained in the red corpuscles.

A further proof of the chemical combination of oxygen was obtained when Bernard [3] and Hoppe-Seyler [4] discovered that the oxygen of the blood could be displaced by an equal volume of carbon monoxide, a gas which formed a more stable combination with the blood. The most convincing proof, however, was furnished when Hoppe-Seyler succeeded in crystallising hæmoglobin, and showed that it combined with oxygen, but yielded up the gas to a vacuum; he also showed that the hæmoglobin, for so he named the pigment of the red corpuscles, had a definite spectrum. A year or two later, in 1864, Stokes [5] discovered that reducing substances removed oxygen from the hæmoglobin and effected a marked change in its colour and spectrum.

The physical and chemical properties of hæmoglobin are described fully in another part [6] of this work; here it is only necessary to discuss the part which the pigment plays in the processes of respiration.

The coefficient of absorption of blood for oxygen is a little lower than that of water, for the presence of salts in solution diminishes the capacity of the liquid to absorb gases.[7] The following table shows the volume of

Temperature.	Oxygen Absorbed according to Different Observers.		
	Bunsen.[8]	Winkler.[9]	Hüfner.[10]
0°	0·04114	0·04890	...
5°	0·03628	0·04286	...
10°	0·03250	0·03802	...
15°	0·02989	0·03415	...
20°	0·02838	0·03103	0·02844
25°	...	0·02844	0·02745
30°	...	0·02616	0·02635
40°	...	0·02306	0·02447
50°	...	0·02090	...

[1] "Die Gase des Blutes," Diss., Göttingen, 1857; *Ztschr. f. rat. Med.*, Bd. viii. S. 256.

[2] *Ann. d. sc. nat.*, Paris, 1857, Sér. 4, Zool., tome viii. p. 125; *Journ. de physiol. expér.*, Paris, 1860, tome iii.

[3] "Leçons sur les effets des substances toxiques et médicamenteuses," Paris, 1857, p. 184; "Leçons sur les liquides de l'organisme," Paris, 1859, tome i. p. 365; tome ii. p. 427.

[4] *Virchow's Archiv*, Bd. xi. S. 288; Bd. xiii. S. 104.

[5] *Proc. Roy. Soc. London*, vol. xiii. p. 357.

[6] Article "Hæmoglobin," this Text-book, vol. i.

[7] Mackenzie, *Ann. d. Phys. u. Chem.*, Leipzig, 1876, Bd. i. S. 438; Setschenow, *Ztschr. f. physikal. Chem.*, Leipzig, 1889, Bd. iv. S. 117; Hüfner, *Arch. f. Physiol.*, Leipzig, 1894, S. 130; 1895, S. 209.

[8] *Ann. d. Chem. u. Pharm.*, Bd. xciii. S. 1; "Gasometrische Methoden," Braunschweig, 1857, S. 136.

[9] *Ztschr. f. physikal. Chem.*, Leipzig, 1892, Bd. ix. S. 174.

[10] *Ann. d. Phys. u. Chem.*, Leipzig, 1876, Bd. i. S. 632; *Arch. f. Physiol.*, Leipzig, 1890, S. 27.

oxygen, measured at 0° and 760 mm., which can be absorbed by one volume of water.

Bohr[1] found that the absorption coefficient of oxygen in a 2 per cent. aqueous solution of hæmoglobin at 15° was 0·02249, whereas that for pure water is, according to Winkler, 0·03415, or 50 per cent. greater at the same temperature.

It has been shown that arterial blood with a temperature of 37° contains a large quantity of oxygen, about 22 volumes per cent., an amount which could not be present in simple solution. Further, when the red corpuscles are absent, as in plasma and serum, the amount of oxygen in the fluid is, according to Pflüger,[2] only 0·26 vols. per cent.

In the next place, different observers have shown that crystals of hæmoglobin can absorb large quantities of oxygen.

Condition of Hæmoglobin.	Amount of Oxygen (0° and 760 Mm.).	Observer.
100 grms. moist crystals	108·4 c.c.	Hoppe-Seyler.[3]
,, crystals dried with filter-paper . .	76·9 ,,	,,
,, crystals dried at 0° and powdered .	54·2 ,,	,,
,, ,, ...	156·6 ,,	Dybkowsky.[4]
,, ,, from horse	44·83–88·52 ,,	Strassburg.[5]
,, ,, ...	180·3 ,,	Preyer.[6]
,, ,, ...	172·4 ,,	,,
,, ,, ...	128 ,,	Worm Müller.[7]
,, ,, ...	159 ,,	Hüfner.[8]
Mean of 10 determinations. ,, crystals dried at 0° and powdered, from ox-blood	134 ,,	Hüfner.[9]

The causes of these differences are various; the crystals of hæmoglobin were prepared in different ways, and it is probable that in some cases methæmoglobin or other products were formed; the amount of moisture varied, and the methods employed for the extraction of the oxygen were different.

Bohr,[10] however, would explain these differences in another manner, for he maintains that there are at least four different kinds of hæmoglobin, α-, β-, γ-, and δ-hæmoglobin, which have the same spectrum, but combine with different amounts of oxygen, 0·4, 0·8, 1·7, and 2·7 c.c.

[1] "Exp. Untersuch. u. d. Sauerstoffaufnahme des Blutfarbstoffes," Copenhagen, 1885, S. 37.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 73.
[3] *Virchow's Archiv*, Bd. xxix. S. 598 ; *Med.-chem. Untersuch.*, 1867, Bd. ii. S. 191.
[4] Hoppe-Seyler, *Med.-chem. Untersuch.*, 1866, Bd. i. S. 117.
[5] *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 454.
[6] *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, No. 21.
[7] *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, Leipzig, 1870, Bd. xxii. S. 351.
[8] *Ztschr. f. physiol. Chem.*, Strassburg, 1878, Bd. i. S. 317, 386.
[9] *Arch. f. Physiol.*, Leipzig, 1894, S. 130.
[10] *Skandin. Arch. f. Physiol.*, Leipzig, 1892, Bd. iii. S. 47, 69, 76, 101.

of oxygen for 1 grm. of hæmoglobin. The usual form of hæmoglobin is γ-hæmoglobin; this, when dried, gives a crystalline powder, α-hæmoglobin, which in turn yields, on solution in water, β-hæmoglobin. A solution of γ-hæmoglobin, when kept in a closed tube, is converted into δ-hæmoglobin. These various kinds of hæmoglobin have different "specific oxygen capacities," by which term Bohr designates the ratio between the number of grammes of iron and the number of cubic centimetres of oxygen present in a given volume of blood, of blood corpuscles or solutions of hæmoglobin, saturated with air at ordinary pressure and temperature. The red blood corpuscles are said to undergo alterations in their specific oxygen capacity during their passage through the circulation.

These results and theories have been subjected to an experimental examination by Hüfner,[1] who maintains that in fresh, healthy ox-blood there is only one kind of hæmoglobin, that the capacity of the fresh hæmoglobin for carbon monoxide and for oxygen is the same, whether it be hæmoglobin directly dissolved from red corpuscles or hæmoglobin first crystallised and then dissolved in water. By experiment, Hüfner shows that 1 grm. of hæmoglobin takes up 1·338 c.c. of carbon monoxide or oxygen measured at 0° and 760 mm. This is confirmed by the following facts. The capacity of hæmoglobin to combine with oxygen appears to depend upon its iron, one atom of which holds two atoms of oxygen. The hæmoglobin of ox-blood contains 0·336 per cent. of iron, and its molecular weight is 16,669; its capacity for carbon monoxide or oxygen, as calculated from its percentage of iron, is 1·34 c.c. for 1 grm., a figure practically identical with that obtained by direct experiment.[2] This is probably also the case with hæmoglobin obtained from the horse, dog, pig, rabbit, and fowl, for Bunge and others[3] have shown that the general percentage of iron is 0·335 per cent. Further, the amount of hæmoglobin in human blood is about 14 per cent., and since 1 grm. of hæmoglobin can absorb about 1·34 c.c. of oxygen, it follows that the amount of oxygen combined in arterial blood should be about 20 volumes per cent., and actual experiment shows that this is the case.[4]

It is probable that some of Bohr's results are due to mixtures of pure and partly decomposed hæmoglobin, and that some of the hæmoglobin may be in the form of methæmoglobin. The same criticism may possibly apply to the results obtained by Haldane and Lorrain Smith.[5]

The oxygen in the blood of invertebrates.—In many of the invertebrate animals, hæmoglobin, hæmocyanin, and other proteids, which can enter into loose combination with oxygen, are found and play a part in the process of respiration. It is impossible, however, in a few words, to do justice to this interesting portion of comparative physiology; for further details, the article by Halliburton[6] on the blood of invertebrate animals should be consulted.

[1] *Arch. f. Physiol.*, Leipzig, 1894, S. 130.

[2] See also Hoppe-Seyler, *Virchow's Archiv*, Bd. xxix. S. 598; *Med.-chem. Untersuch.*, 1867, Bd. ii. S. 191; Preyer, "De hæmoglobino observationes et experimenta," Bonnæ, 1866, p. 19; *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, No. 21.

[3] Jaquet, *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiv. S. 289.

[4] See p. 761.

[5] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 468.

[6] "Text-Book of Chemical Physiology and Pathology," London, 1891, pp. 316–330. Here numerous references to previous work on the subject will be found. Among subsequent papers may be mentioned those of Griffiths, *Compt. rend. Acad. d. sc.*, Paris, 1892, tome cxv. pp. 259, 419, 474, 669, 738; cxvi. p. 1206.

Nitrogen.—The blood contains about 1·8 volumes per cent. of nitrogen, and this is present chiefly in a condition of solution. Thus Lothar Meyer[1] and others[2] have found that the absorption of nitrogen by defibrinated blood is proportional to the pressure. This, Paul Bert[3] showed, was also the case in living animals, but, owing to the want of perfect ventilation of the lungs, the increase did not exactly follow Dalton's law. Thus—

Pressure in Atmospheres.	Percentage of Nitrogen in Dog's Blood.	Pressure in Atmospheres.	Percentage of Nitrogen in Dog's Blood.
1	2·2	5	6·0
2	3·0	7	7·0
3	3·9	10	9·4

The coefficient of absorption of water for nitrogen is small, and the blood has even less power of absorption, for Fernet, Setschenow, Hüfner, and others[4] have shown that the presence of other substances in solution diminishes the capacity of water to absorb gases. The following table shows the coefficient of absorption of water for nitrogen at different temperatures :—

Temperature.	Coefficient of Absorption.		
	Bunsen.[5]	Hüfner.[6]	Winkler.[7]
0°	0·02035	...	0·02348
5°	0·01794	...	0·02081
10°	0·01607	...	0·01857
15°	0·01478	...	0·01682
20°	0·01403	0·01406	0·01542
25°	...	0·01357	0·01431
30°	...	0·01308	0·01340
37°	...	0·01239	...
40°	...	0·01210	0·01183

[1] "Die Gase des Blutes," Inaug. Diss., Göttingen, 1857, S. 56.

[2] Setschenow, *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, Bd. xxxvi. S. 293.

[3] "La pression barométrique," Paris, 1878, p. 661.

[4] Fernet, *Ann. d. sc. nat.*, Paris, Sér. 4, "Zool.," tome viii. p. 125 ; Setschenow, *Mém. Acad. imp. d. sc. de St. Pétersbourg*, 1879, tome xxvi. p. 6; *Ztschr. f. physikal. Chem.*, Leipzig, 1889, Bd. iv. S. 117 ; Hüfner, *Arch. f. Physiol.*, Leipzig, 1894, S. 130 ; 1895, S. 209 ; Mackenzie, *Ann. d. Phys. u. Chem.*, Leipzig, 1876, Bd. i. S. 438 ; Bohr, "Exper. Untersuch. u. d. Sauerstoffaufnahme des Blutfarbstoffes," Copenhagen, 1885, S. 37.

[5] "Gasometrische Methoden," 1857, S. 136 ("Gasometry," Roscoe's transl., London, 1857).

[6] *Ann. d. Phys. u. Chem.*, Leipzig, 1877, Bd. i. S. 632 ; *Arch. f. Physiol.*, Leipzig, 1890, S. 27.

[7] *Ztschr. f. physikal. Chem.*, Leipzig, 1892, Bd. ix. S. 173.

Further proofs that the nitrogen is simply in solution are afforded by two experiments made by Pflüger. Blood subjected to the vacuum of a mercurial pump quickly gives off its nitrogen; thus at 0° all the nitrogen, but less than half the oxygen and three-quarters of the carbon dioxide, were given off in twenty hours.[1] The blood of a dog which had previously breathed for a few minutes a mixture containing only oxygen and carbon dioxide, yielded no nitrogen to a vacuum; that gas had rapidly diffused from the blood into the air of the lungs.[2]

Carbon dioxide.—The nature of the connection between the carbon dioxide and the blood, which contains it, is very difficult to follow, and has given rise to much discussion.[3] There is no single substance with which the whole of the carbon dioxide is combined; it is present both in the red corpuscles and plasma, and, after coagulation of the blood, in both the clot and serum. It will be well, therefore, to consider—(1) The amount of this gas, which may be in a state of simple solution in the blood and in serum; (2) the quantity in loose and firm chemical combination with substances in the corpuscles and in the plasma and serum of the blood.

Carbon dioxide is much more soluble in water than oxygen and nitrogen. Plasma and serum are not able to retain in simple solution as much carbon dioxide as can a similar volume of pure water, for it has already been mentioned that the presence of indifferent substances in solution diminishes the capacity of the fluid to absorb gases. There are, however, exceptions[4] to this general rule, and it is therefore necessary to determine experimentally the absorption coefficient of carbon dioxide in blood before we conclude that it is less than in water. This experiment was made by Zuntz,[5] who neutralised the blood with phosphoric or oxalic acid in order to eliminate its chemical affinity, saturated it with carbon dioxide, and then determined the amount absorbed. He found that the coefficient of absorption for calves' blood with a specific gravity of 1038 was 1·626, and that for sheep's blood with a specific gravity of 1052 was 1·547 at 0°.

Partial Pressure of CO_2 in the Mixture of Gases Used.		Carbon Dioxide in Dog's Serum (0° and 760 Mm.).		
Mm. mercury.	Percentage of an Atmosphere.	Total.	Quantity Absorbed.	Quantity in Chemical Combination.
105·8	13·9	61·1 per cent.	20·7 per cent.	46·4 per cent.
351·4	46·2	122·1 ,,	68·8 ,,	53·3 ,,
747·8	98·4	202·2 ,,	146·4 ,,	55·8 ,,

[1] Pflüger, "Die Kohlensäure des Blutes," Bonn, 1864, S. 12.

[2] Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 104.

[3] For further details see Zuntz, Hermann's "Handbuch," Bd. iv. Th. 2, S. 64; Hammarsten, "Lehrbuch der physiologischen Chemie," Wiesbaden, 1895, S. 535; Setschenow, *Mém. Acad. imp. d. sc. de St. Pétersbourg*, 1879, tome xxvi. p. 6; Zuntz, "Beitr. z. Physiologie des Blutes," Inaug. Diss., Bonn, 1868, S. 33.

[4] Buchanan, *Proc. Roy. Soc. London*, 1874, No. 15, p. 192.

[5] "Beitr. z. Physiol. des Blutes," S. 39; Hermann's "Handbuch," Bd. iv. Th. 2, S. 15.

Setschenow[1] calculated that serum held in simple solution 99 per cent. of the amount of carbon dioxide which distilled water would absorb under similar conditions, and that one-tenth of the total carbon dioxide in the serum of dog's blood was in simple solution. The result of further experiments made by Zuntz[2] upon these points is shown in the table on p. 770.

In the next place, it is necessary to consider the amount of carbon dioxide in loose and firm chemical combination with substances in the corpuscles, plasma and serum. Most of the gas is contained in the plasma or serum, for these fluids contain a larger quantity of carbon dioxide than that which can be obtained from an equal volume of blood. The greater quantity of the gas is in a state of loose chemical combination in the serum, for much of it can be extracted by the action of the vacuum of a blood-pump; the remainder, however, is in firm chemical combination, and is only set free in the pump by the addition of an acid. In this respect a marked contrast is observed between blood and serum, for *all* the carbon dioxide can be extracted from the former by the action of the vacuum alone, the hæmoglobin of the red corpuscles playing, apparently, the part of an acid.[3]

The following table shows the amount of carbon dioxide in loose and firm chemical combination in serum:—

Carbon Dioxide in Serum. (Volumes per cent.)			Carbon Dioxide in Blood. (Volumes per cent.)	Observer.
Extracted by Vacuum.	In Firm Combination.	Total in Combination.		
13·4	31·3	44·7	34·5	Schœffer.[4]
21·1	21·9	43·0	35·0	,,
44·6	4·9	49·5	...	Pflüger.[5]
35·2	9·3	44·5	...	,,
19·9	6·9	26·8	...	Zuntz.[6]
22·0	12·4	34·4	...	,,
22·5	13·5	36·0	...	,,
26·9	17·0	43·9	...	,,

The differences in these results are due, as Zuntz[7] has pointed out, to the powerful action of Pflüger's pump, and to the concentration of the serum during its exposure to the vacuum. The carbonates of the serum give off their gas more readily when the solution is concentrated; this complication

[1] *Loc. cit.*, *Centralbl. f. d. med. Wissensch.*, Berlin, 1877, No. 35.

[2] Hermann's "Handbuch," Bd. iv. Th. 2, S. 68.

[3] Setschenow, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1859, Bd. xxxvi. S. 293; Pflüger, "Ueber die Kohlensäure des Blutes," Bonn, 1864, S. 5; Zuntz, *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 527.

[4] *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1860, Bd. xli. S. 616.

[5] "Ueber die Kohlensäure des Blutes," Bonn, 1864, S. 11.

[6] *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 529; Hermann's "Handbuch," Bd. iv. Th. 2, S. 45.

[7] *Loc. cit.*

was avoided in the analyses made by Zuntz, by the addition of distilled water in sufficient quantity to maintain the concentration of the fluid at its original point. Preyer[1] found that the proportion of carbon dioxide in loose and in firm combination was as 2 to 3·5.

The next question to discuss is the nature of the substances with which the carbon dioxide is combined. The facts already mentioned show that these substances are to be sought chiefly in the serum. In the first place, analyses of the ash of serum show that the most important constituents are the alkalies; thus, according to Bunge's[2] experiments, the ash from 1000 grms. of dog's serum contains 4·341 grms. sodium, of which 3·463 grms. is sufficient to saturate the chlorine. The remainder, 0·878 grms. sodium, can combine with 0·623 grms. carbon dioxide (316 c.c. at 0° and 760 mm.) to form sodium carbonate, and, in addition, with another equal quantity to form sodium bicarbonate. Thus calculated, a litre of plasma could hold 632 c.c. of carbon dioxide, or 63 volumes per cent. in chemical combination. This must be considered only as an approximate result, for the amount of sodium carbonate in serum cannot be accurately determined by an analysis of the ash or by titration, for the alkali is combined with other substances, especially with proteids.[3]

The alkalies of the blood are the most important constituents for holding carbon dioxide in combination. Serum freed from gas can combine with as much carbon dioxide as is necessary to form bicarbonates with its alkalies; any reduction in the alkalinity of the blood is accompanied by a decrease in carbon dioxide. Thus Walter[4] found only 2 to 3 volumes per cent. of carbon dioxide in the blood of rabbits poisoned by hydrochloric acid; Geppert and Zuntz[5] observed that the alkalinity of the blood of rabbits was diminished by the acid formed during tetanic muscular activity, and at the same time there was a decrease in the carbon dioxide of the blood. During diabetic coma the alkali of the blood appears to be in great part neutralised by combination with β-oxybutyric acid;[6] and Minkowski[7] found only 3·3 volumes per cent. of carbon dioxide in the blood of a patient suffering from diabetic coma.

Another substance with which the carbon dioxide is supposed to combine in serum is disodium hydrogen phosphate[8] (Na_2HPO_4), with the formation of sodium bicarbonate and sodium biphosphate. Thus—

$$Na_2HPO_4 + CO_2 + H_2O = NaHCO_3 + NaH_2PO_4.$$

Sertoli[9] and Mroczkowski,[10] however, found that the quantity of phosphoric acid in the serum is so small that, if allowance be made for that contained in lecithin and nuclein, the amount is quite insufficient

[1] *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, Bd. xlix. S. 27.

[2] *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 204; "Lehrbuch der physiologischen und pathologischen Chemie," Leipzig, 1889, S. 254.

[3] Hoppe-Seyler, "Physiol. Chem.," Berlin, 1879, Bd. iii. S. 502; Sertoli, *Med.-chem. Untersuch.*, Berlin, 1868, Heft 3, S. 350.

[4] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. vii.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 189. See also this article, p. 714.

[6] Stadelmann, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. vii.; Minkowski, *ibid.*, Bd. xviii.; *Mitth. a. d. med. Klin. zu Königsberg*, Leipzig, 1888.

[7] *Loc. cit.*

[8] Fernet, *Ann. d. sc. nat.*, Paris, Sér. 4, tome viii. p. 160; Heidenhain and L. Meyer, *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1863, Heft 2; *Ann. d. Chem. u. Pharm.*, 1862–63, Supp. Bd. ii. S. 157.

[9] Hoppe-Seyler, *Med.-chem. Untersuch.*, Berlin, 1868, Heft 3, S. 350.

[10] *Centralbl. f. d. med. Wissensch.*, Berlin, 1878, No. 20, S. 356.

to play any important part in combining with carbon dioxide. Bunge,[1] on the other hand, maintains that in dog's blood the quantity of phosphoric acid is sufficient, and that only a small quantity is combined with alkalies in the plasma; he agrees, however, with the previous observers, that the amount of phosphoric acid in the blood of the ox and the pig is very small.

There is also evidence to show that the proteids, especially the globulin of serum, play some part in forming combinations with carbon dioxide. Setschenow[2] considered that the globulin formed a combination with the carbon dioxide, whereas Sertoli held that the globulin acted as an acid, and in the serum was combined with an alkali.

The blood corpuscles contain about one-third of the total carbon dioxide found in the blood.[3] The gas is in loose chemical combination probably with the alkali of the phosphates, globulin, and hæmoglobin of the corpuscles, and directly with the hæmoglobin. Setschenow calculates that in 100 volumes of blood the red corpuscles contain 10 volumes, and the white corpuscles 2·5 volumes of carbon dioxide.

The experiments of Setschenow,[4] Zuntz,[5] Bohr,[6] and Torup[7] show that carbon dioxide combines with hæmoglobin even in the absence of an alkali. A solution of pure crystallised hæmoglobin absorbs more carbon dioxide than does an equal volume of water, and the amount of gas absorbed is relatively large for low pressures, but relatively small for high pressures. According to Bohr, 1 grm. of hæmoglobin at 18°·4, and under a pressure of 30 mm., combines with 2·4 c.c. of carbon dioxide; the pigmented portion of the hæmoglobin is supposed to combine with oxygen and the proteid portion with carbon dioxide.

Further investigation, however, is necessary before it will be possible with any exactitude to decide the relative importance of the different combinations with the carbon dioxide of the blood.

The causes of the exchange of gases between the air in the lungs and the blood.—The oxygen of the blood is derived from the air in the alveoli of the lungs; the carbon dioxide in the expired air comes from the pulmonary blood, and ultimately from the tissues of the body. The inspired air contains at 0° and 760 mm. 20·96 volumes per cent. of oxygen, the expired air about 16 per cent., and the tissues no free oxygen; the carbon dioxide is 0·03 volumes per cent. in the inspired air, about 4 in the expired air, and in the tissues is being constantly produced. There would, therefore, appear to be sufficient causes, both physical and chemical, to determine the passage of the oxygen inwards and of the carbon dioxide outwards.

Oxygen,	Alveolar air	⟶	Blood	⟶ Tissues.
Carbon Dioxide,	Tissues	⟶	Blood	⟶ Alveolar air.

[1] *Ztschr. f. Biol.*, München, 1876, Bd. xii. S. 206; "Lehrbuch der physiologischen und pathologischen Chemie," Leipzig, 1889, S. 256.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. viii. S. 1; *Centralbl. f. d. med. Wissensch.*, Berlin, 1877, No. 35; 1879, No. 21; *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1879, Bd. xii. S. 855; *Mém. Acad. imp. d. sc. de St. Pétersbourg*, 1879, tome xxvi. No. 13.

[3] Alex. Schmidt, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Cl.*, Leipzig, 1867, Bd. xix. S. 30; Zuntz, *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 529; Hermann's "Handbuch," Bd. iv. Th. 2, S. 72; Fredericq, "Recherches sur la constitution du plasma sanguin," Gand, 1878, p. 49.

[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1877.

[5] Hermann's "Handbuch," Bd. iv. Th. 2, S. 76.

[6] *Beitr. z. Physiol. Carl Ludwig z. s. 70 Geburtst.*, Leipzig, 1887, S. 164; *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xvii. S. 115.

[7] *Jahresb. ü. d. Fortschr. d. Thier-Chem.*, Wiesbaden, Bd. xvii. S. 115.

The evidence, however, in support of this explanation must be examined, for of late it has been challenged, especially by Bohr.[1] In the first place, it is necessary to remember that the composition of the alveolar air is not represented by that of the air expired. The composition of the inspired and of the expired air and the tension of their component gases can be readily determined. The tension of oxygen in the inspired air is 159 mm., under the mean pressure of an atmosphere, 760 mm. It is difficult, however, to obtain with accuracy similar data for the air of the alveoli. From the numerous analyses of expired air in a man, it is possible to form only a rough estimate of the alveolar air; it probably contains 5 to 6 per cent. of carbon dioxide, and 14 to 15 per cent. of oxygen; and the tension of the former would be about 36 mm., and of the latter about 114 mm. Löwy[2] calculates that the tension of oxygen in the alveoli of the human lungs is from 12·6 to 13·5 per cent. of an atmosphere, or about 99 mm. of mercury.

In animals, direct determinations of the composition of the alveolar air of an occluded portion of the lungs have been made. For the collection of this air Pflüger[3] constructed a special catheter (Fig. 71). It consists of an ordinary fine elastic catheter, surrounded, except at its extremities, by a tube with a rubber enlargement towards the free end of the catheter. The instrument is so small that, when introduced through the trachea into a bronchus of a dog, it causes no hindrance to the free passage of air into the other parts of the lungs. The rubber enlargement is now inflated, and shuts off a portion of the lungs, from which the alveolar air can be withdrawn through the inner tube of the lung catheter. In such experiments Wolffberg[4] and Nussbaum[5] found that the alveolar air of a dog contained 3·5 per cent. of carbon dioxide, whereas the expired air yielded 2·8 volumes per cent. It is to be noted that this value for the alveolar air is higher than the normal, for the air in the alveoli was shut off from the tidal air, and, in fact, represents the air after an equilibrium had been established with the gases of the blood passing through that portion of the lung shut off by the catheter.

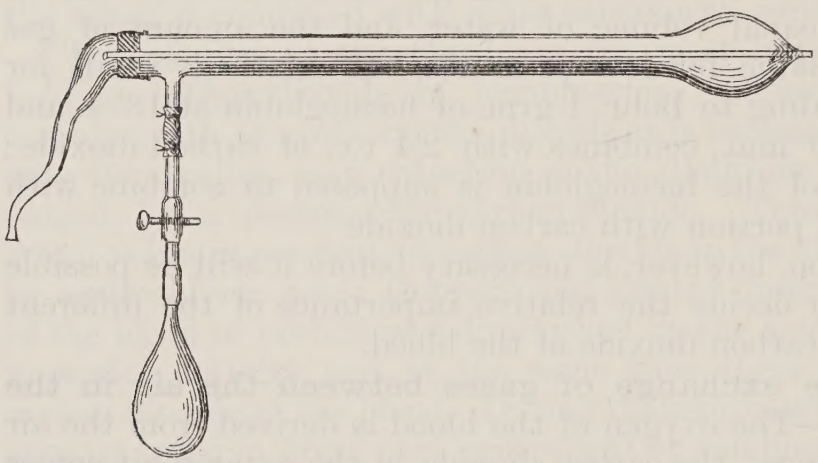


Fig. 71.—Pflüger's lung catheter.

In the next place, it is necessary to consider the tension of the oxygen and carbon dioxide present in the blood, and this involves a preliminary study of the *dissociation* of oxyhæmoglobin. Under the ordinary tension of oxygen in the air, hæmoglobin readily combines with oxygen, but if the external pressure be lowered sufficiently, then oxygen is given off, and the oxyhæmoglobin undergoes dissociation.

[1] *Skandin. Arch. f. Physiol.*, Leipzig, 1891, Bd. ii. S. 236.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 416; "Untersuch. u. d. Respiration und Circulation," 1895, S. 26.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 43.
[4] *Ibid.*, 1871, Bd. iv. S. 465; 1872, Bd. vi. S. 23.
[5] *Ibid.*, 1873, Bd. vii. S. 296.

The force with which the oxygen separates from the hæmoglobin under these circumstances is called the *tension of dissociation.* The most important researches upon this subject are those of Hüfner.[1]

The conditions of the dissociation of oxyhæmoglobin are the same, whether it is a solution of freshly-made pure crystals of hæmoglobin, or fresh defibrinated blood. The dissociation is dependent upon the concentration of the solution of hæmoglobin; thus, a weak solution is more readily dissociated under a given pressure than a strong solution. It is also affected by temperature.[2] As regards pressure, Hüfner found in the case of a solution containing 14 per cent of oxyhæmoglobin at 35°, that, under a tension of oxygen of 152 mm., 98·42 per cent. of the pigment was oxyhæmoglobin, and 1·58 per cent. hæmoglobin. When the tension of oxygen was reduced to 75 mm., the percentages of oxyhæmoglobin and of hæmoglobin were respectively 96·89 and 3·11, and with a lower pressure the dissociation became more rapid, as shown by the following curves:—

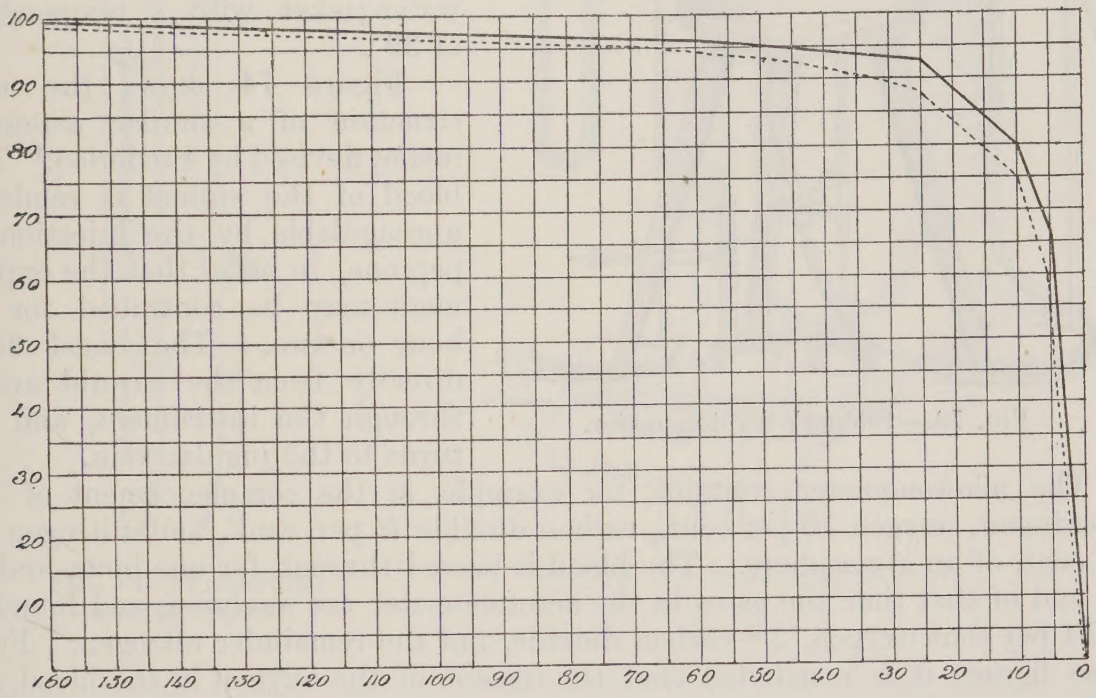


FIG. 72.—Curves of dissociation of oxyhæmoglobin. The continuous line is for a solution containing 14 per cent. of hæmoglobin, the interrupted line for a 4 per cent. solution.

It is now necessary to compare with the tension of the oxygen and carbon dioxide in the alveolar air the tension of those gases in the blood. For the determination of these tensions in blood Pflüger[3] used a special instrument, known as the aërotonometer (see Fig. 73).

The principle of the aërotonometer and of other similar instruments is this: Blood in contact with a mixture of oxygen, nitrogen, and carbon dioxide gives up some of its gases if their partial pressures are greater than those of the corresponding gases in the mixture; on the other hand, if the tensions of the gases in the blood be lower than the respective tensions of the gases in the mixture, the blood takes up gas. These interchanges persist until equilibrium is established, until the tension or partial pressure of the gas in the blood is

[1] *Ztschr. f. physiol. Chem.*, Strassburg, Bd. vi. S. 109; Bd. xii. S. 582; Bd. xiii. S. 285; *Arch. f. Physiol.*, Leipzig, 1890, S. 1; *ibid.*, 1895, S. 213.

[2] Brasse, *Compt. rend. Soc. de biol.*, Paris, 1888, S. 660.

[3] Described by Strassburg, *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 65.

equal to that of the corresponding gas in the mixture. In the aërotonometer the blood is made to pass in a thin layer through a glass tube or tubes, containing mixtures of gases of known quantity and tension, and it is arranged by practice that the tension of the gases in the tubes shall in the one case be greater, in the other case smaller, than the tensions of the corresponding gases in the blood. The gases in these tubes, after the blood has passed through them, are analysed, and from the alteration in the proportion in the two tubes it is possible to calculate the partial pressure of the gases in the blood. The aërotonometer is surrounded by a water-jacket with a temperature of 39°.

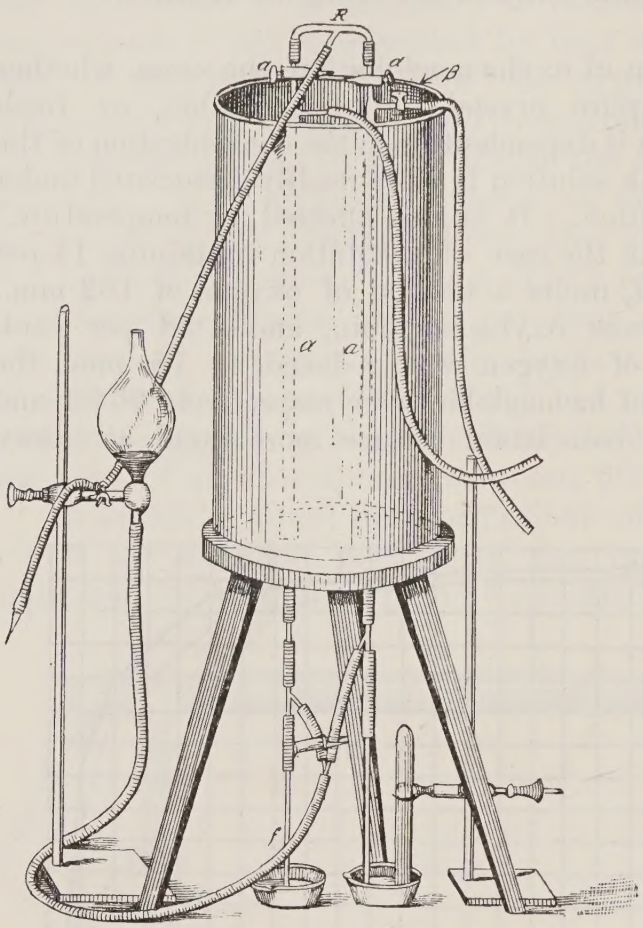


FIG. 73.—Pflüger's aërotonometer.

Figure 74 shows the construction of a similar aërotonometer, devised by Fredericq.[1] The blood of the animal is rendered uncoagulable by the injection of peptone, in order that the experiment may be continued for an hour or two. The blood flows directly from the carotid artery through the instrument, and returns to the jugular vein.

The aërotonometer contains, for example, at the commencement of the experiment, oxygen 10 per cent., carbon dioxide 5 per cent., and nitrogen 85 per cent. of an atmosphere. The blood is passed through for one hour, and at the end of that time the gases in the aërotonometer are analysed, and found to be 14 per cent oxygen, 2·8 carbon dioxide, and the remainder nitrogen. From these figures it is concluded that the tension of the oxygen in the blood was 14 per cent. of an atmosphere, and that of the carbon dioxide 2·8 per cent. of an atmosphere.

Bohr[2] had previously introduced a modified aërotonometer, the "hæmat-aërometer," through which a constant and rapid stream of blood could be maintained during each experiment (see Fig. 75).

What, then, are the tensions of the gases of the blood? The results obtained by different observers are very discordant, and have given rise to considerable discussion.[3] Nussbaum[4] determined simultaneously on a dog the tension of the carbon dioxide in the blood from the right side of the heart and in the air of the alveoli; he found for the former a pressure of 3·81 per cent. of an atmosphere, and for the latter 3·84 per cent. The tension of the carbon dioxide in normal alveolar air would be lower, for it would be mixed to a certain extent with the

[1] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, S. 33; Fredericq et Nuel, "Eléments de physiologie humaine," 3e édition, 1893, p. 156.

[2] *Skandin. Arch. f. Physiol.*, Leipzig, 1891, Bd. ii. S. 238.

[3] Bohr, *loc. cit.*; Fredericq, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, S. 33; Haldane and Lorrain Smith, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 497.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii. S. 296.

tidal air. Wolffberg[1] found that the expired air of a dog contained 2·8 volumes per cent. of carbon dioxide, or a tension of 21·3 mm. of mercury. Strassburg[2] found a tension of 5·4 per cent. of an atmosphere for the

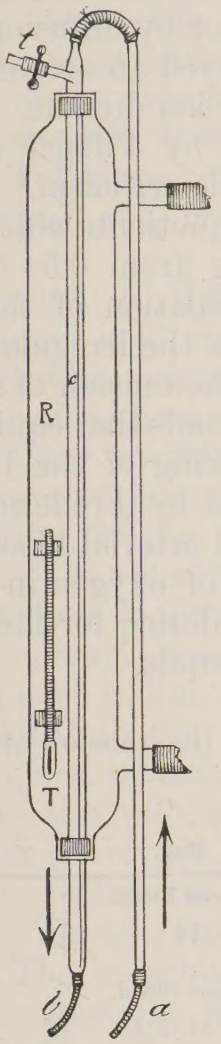


Fig. 74.—Fredericq's aërotonometer.

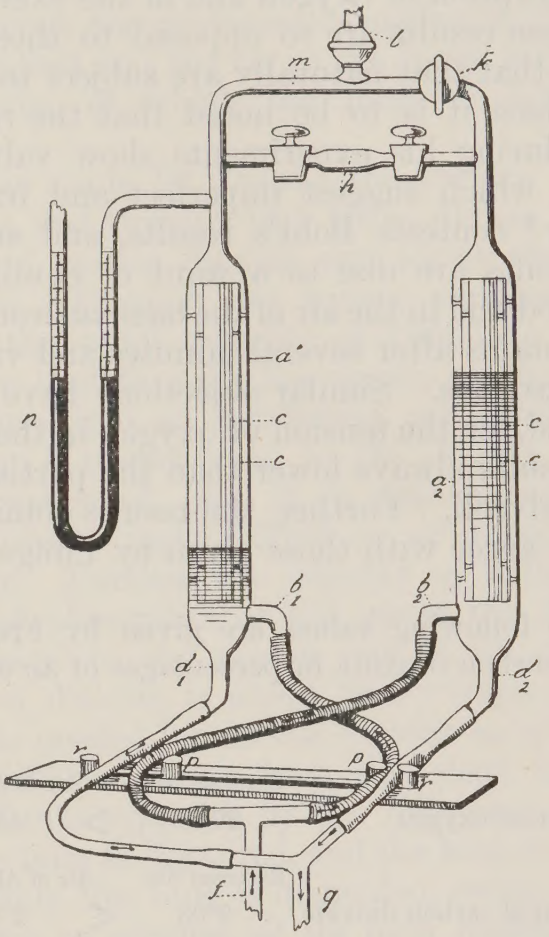


Fig. 75.—Bohr's hæmataërometer.

carbon dioxide in the venous blood of the right side of the heart. This value, higher than those obtained by Wolffberg and Nussbaum, could be explained by the fact that the dog's lungs were not so well ventilated, since tracheotomy had not been performed. In arterial blood Strassburg found the tension of carbon dioxide to be 2·2 to 3·8 per cent. of an atmosphere, and for the oxygen Herter[3] obtained a tension of 10 per cent. of an atmosphere.

Very different results have been obtained by Bohr[4] in experiments upon dogs. He obtained for the oxygen tension of arterial blood results as high as 101 to 144 mm. of mercury, and in nearly every case the tension was higher than the tension of oxygen in the air at the bifurcation of the trachea, in one case by as much as 38 mm. As regards the tension of carbon dioxide very discordant results were obtained. In eleven experiments, in which the animal breathed pure air, the tension of the carbon dioxide in the arterial blood varied between 0 and 28 mm. of mercury; and in five other experiments, when the air inspired contained carbon dioxide,

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 478.
[2] *Ibid.*, 1872, Bd. vi. S. 77.
[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1879, Bd. iii. S. 98.
[4] *Skandin. Arch. f. Physiol.*, Leipzig, 1891, Bd. ii. S. 236.

the tension of that gas varied between 0·9 and 57·8 mm. In the majority of the experiments the air of the trachea contained carbon dioxide with a higher tension than that of the gas in the blood. From these results Bohr concluded that the exchange of gases between the air of the alveoli and the blood in the lungs could not be accounted for by diffusion alone, and he suggested that the tissues of the lungs played an active part in the absorption of oxygen and in the excretion of carbon dioxide.

These results are so opposed to those obtained by Pflüger and his pupils, that they naturally are subject to considerable criticism.[1] In the first place, it is to be noted that the respiratory quotients obtained by Bohr during his experiments show values varying from 0·54 to 1·01, results which suggest imperfect and irregular ventilation of the lungs. Hüfner[2] contests Bohr's results, and suggests that the irregularities in the results are due to a want of equilibrium in the tension of gases in the blood and in the air of the hæmataërometer. He finds that equilibrium only obtains after several minutes and vigorous shaking of the blood in the apparatus. Similar objections have been made by Fredericq,[3] who obtained, for the tension of oxygen in the peptonised arterial blood of the dog, results always lower than the partial pressure of oxygen in the air of the alveoli. Further, the results obtained by Fredericq for the carbon dioxide agree with those given by Pflüger and his pupils.

The following values are given by Fredericq[4] for the tension of oxygen and of carbon dioxide in percentages of an atmosphere.

				Dog.			
	External Air.		Air of Alveoli.		Arterial Blood.		Tissues.
Tension of oxygen	20·95	>	18	>	14	>	0
	External Air.		Air of Alveoli.		Venous Blood.		Tissues.
Tension of carbon dioxide	0·03	<	2·8	<	3·81–5·4	<	5–9

Quite recently Haldane and Lorrain Smith[5] have studied the tension of oxygen in the arterial blood of man by a new method, which, they maintain, avoids the probable sources of fallacy in the aërotonometer. In this new method the tension of oxygen in the arterial blood is calculated from the percentage of carbon monoxide breathed by the subject of the experiment, and from the final saturation of his blood with carbon monoxide. The results give, for the oxygen tension of human arterial blood, a value of 26·2 per cent. of an atmosphere, or 200 mm. of mercury. This value is about twice as high as that of the oxygen in the pulmonary alveoli, and if it be correct, it follows that diffusion alone does not explain the absorption of oxygen by the blood in the lungs. Haldane and Lorrain Smith discuss some of the possible sources of error in their method, such as the estimation of the saturation of the blood with carbon monoxide, the dissociation of carboxyhæmoglobin, the effect of dilution of the hæmoglobin, and the excretion or oxidation of carbon monoxide; but the test experiments which they made confirm them in their opinion of its accuracy.

Before, however, these results are accepted, further experiments are needed to test the method, for it is impossible with our present knowledge to judge

[1] See also criticism by Zuntz, *Fortschr. d. Med.*, Berlin, 1890, Bd. viii. S. 856.
[2] *Arch. f. Physiol.*, Leipzig, 1890, S. 10.
[3] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, S. 33.
[4] Fredericq et Nuel, "Eléments de physiologie humaine," Gand, 1893, pp. 156–158.
[5] *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 497.

it correctly. Some of the results obtained by Haldane and Lorrain Smith in their examination of these sources of fallacy are opposed to those obtained by Hüfner [1] and Saint-Martin.[2]

It is impossible to pass a verdict upon such discordant evidence, especially since further investigation is necessary to test the soundness of many of the experiments and of the conclusions based upon the results. It is permissible, however, to accept the provisional conclusion that the exchange of gases between the blood and the air in the lungs is effected by physical and chemical means, of which the most important is diffusion.

According to the calculations made by Zuntz,[3] the surface of the human lungs is 90 square metres, and through this there diffuse during quiet breathing about 300 c.c. of carbon dioxide and about the same quantity of oxygen in a minute. Through the square centimetre of surface there would pass only the small quantity of 0·0003 c.c. of gas. Now Exner's [4] experiments show that through the square centimetre of a soap film 0·6 c.c. of air diffuse into an indifferent gas during one minute. The velocity of diffusion is proportional to the density of the gas, therefore a difference in tension of $\frac{1}{2000}$ of an atmosphere, or 0·3 mm. of mercury, would be sufficient to make 0·0003 c.c. of oxygen pass through such a film in a minute. Further, the velocity of diffusion is proportional to the coefficient of absorption of the gas in the fluid in question, and inversely proportional to the square root of its density; therefore the velocity for carbon dioxide is about thirty times greater than that of oxygen, and there is needed for carbon dioxide an even less difference of tension to cause a diffusion of gas from the blood into the alveoli. These considerations Zuntz supports by the following experiment. The bronchus of a frog's lung is ligatured, and the lung is placed in carbon dioxide; within a minute the lung is distended, owing to the diffusion of carbon dioxide being, on account of its high coefficient of absorption, about forty-five times greater than that of air. If a tube be placed in the bronchus, the diffused gas can be collected and measured.

Diffusion appears to be sufficient to account for the phenomena of gaseous exchange in the lungs. Other conditions possibly assist in the process. It has been shown that oxygen in combination with hæmoglobin appears to have the property of driving out carbon dioxide.[5]

Fleischl von Marxow [6] supposes that the sudden percussion given by the contraction of the ventricles to the blood assists in the liberation of the carbon dioxide in the lungs, and of oxygen in the arterioles supplying the tissues of the body. This theory, however, after the criticisms brought forward by Zuntz,[7] appears to be untenable.

[1] *Arch. f. Physiol.*, Leipzig, 1895, S. 213.
[2] *Compt. rend. Acad. d. sc.*, Paris, 1891, tome cxii. p. 1232 ; 1892, tome cxv. p. 835.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 408.
[4] *Ann. d. Phys. u. Chem.*, Leipzig, 1875, Bd. clv. S. 321, 443.
[5] This article, p. 771. See also Holmgren, *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, Bd. xlviii.; Werigo, *Arch. f. d. ges. Physiol.*, Bonn, 1892, Bd. li. S. 321 ; 1892, Bd. lii. S. 194 ; Zuntz, *ibid.*, Bd. lii. S. 191, 198.
[6] "Die Bedeutung des Herzschlages f. d. Athmung, eine neue Theorie des Respiration," Stuttgart, 1887 ; *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, S. 231, 662.
[7] *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 408.

The Exchange of Gases between the Blood and the Tissues. Internal Respiration.

From a comparative study[1] of the process of respiration, it is seen that the exchange of gases in the simplest forms of life is between the external medium and the protoplasm of the cell.

In insects the smallest branches of the tracheal system carry oxygen to the individual cells,[2] which are often the seat of a most energetic combustion. In no case is this more marked than in the luminous organ of the glowworm (*Lampyris splendidula*), where, as Max Schultze[3] has shown, there are special cells at the end of the tracheæ. The phosphorescence still continues after the removal of the organ from the insect's body, and under the microscope is seen to appear first in those parts of the cells which are around the ends of the tracheæ. The luminous cells have a great affinity for oxygen, as shown by the fact that they cease to give out light if confined in an atmosphere free from oxygen,[4] and readily reduce osmic acid.

In the higher animals the blood is the medium which supplies the tissues with oxygen and removes their carbon dioxide and other waste products. Reference has already been made to the theories of Lavoisier and Crawford[5] concerning processes of oxidation in the blood, and we may proceed to consider the experimental evidence which has been advanced in favour of the view, that the blood is the chief seat of combustion. When blood is shed and kept at the temperature of the body, it becomes gradually poorer in oxygen,[6] and there is always a distinct darkening in the colour of arterial blood, even within the first few minutes after it is shed.[7] These changes were investigated by Pflüger in a series of determinations of the gases of the blood, and he found that arterial blood received directly into a large vacuum, surrounded by hot water, gave a percentage of oxygen from 0·2 to 10 per cent. higher than the amount extracted by the slower method of the ordinary gas-pump. About the same time Alexander Schmidt[8] found that when the blood of an asphyxiated animal was exposed to a known quantity of oxygen, there was an absorption and disappearance of oxygen, and an increase in the amount of carbon dioxide. The capacity of blood to bring about this oxidation varied; that taken from contracting muscles could consume from 3 to 4 per cent., that from the heart 2 per cent., and blood from the hepatic vein 0·8 per cent. oxygen. It was shown by Afanassiew[9] that only the blood corpuscles and not the serum could take up oxygen in this way, and Tschiriew[10] found that lymph resembled the serum in containing no reducing substances.

[1] See Paul Bert, "Leçons sur la physiologie comparée de la respiration," Paris, 1870; Johannes Müller, "Elements of Physiology," Baly's trans., vol. i.; Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. x. S. 270.

[2] Finkler, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. x. S. 273; Kupffer, *Beitr. z. Anat. u. Physiol. als Festgabe C. Ludwig*, Leipzig, 1875, S. 67.

[3] *Arch. f. mikr. Anat.*, Bonn, 1865, Bd. i. S. 124.

[4] Milne Edwards, "Leçons sur la physiologie et l'anatomie comparée," tome viii. pp. 93–120.

[5] See p. 756.

[6] Nawrocki, *Stud. d. physiol. Inst. zu Breslau*, Leipzig, Bd. ii. S. 144; Sachs, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1863, S. 348.

[7] Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 61; Bernard, *Journ. de l'anat. et physiol. etc.*, Paris, 1858, tome i. S. 233.

[8] *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Cl.*, Leipzig, 1867, Bd. xix. S. 99; *Centralbl. f. d. med. Wissensch.*, Berlin, 1867, S. 356.

[9] *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, Leipzig, 1872, Bd. xxiv. S. 253.

[10] *Ibid.*, 1874, Bd. xxvi. S. 116.

Alexander Schmidt considered that in the blood an active oxidation took place, for he concluded from his experiments that readily oxidisable substances and active oxygen or ozone existed in that fluid, and further that the oxidation in the body increased with the velocity of the blood. The hæmoglobin was looked upon as the regulator of the consumption of oxygen, and this erroneous view, propounded by Lothar Mayer, is still accepted by some medical writers.

As in all tissues, so in the blood there is a certain amount of oxidation, but the evidence about to be given will show that it is small and unimportant when compared with that taking place in muscles and glands. The blood is not the cause of the oxidation of the body, the cause is in the living cells of the tissues.[1]

The chief evidence is as follows:—A frog can live in an atmosphere of nitrogen for seventeen hours, and during this time gives off carbon dioxide, in fact during the first five hours it discharges as much as it would under normal conditions.[2] A frog will live a day or two in oxygen after its blood has been entirely replaced by normal saline solution,[3] and when in this condition its intake of oxygen and output of carbon dioxide are equal to that of a normal frog.[4] The experiments of Finkler[5] show that the consumption of oxygen is independent, naturally within certain limits, of the velocity of the circulating blood. Further, the respiratory exchange of rabbits, deprived by bleeding of one-half of their hæmoglobin, is equal to that of the same animals before the loss of blood;[6] patients with simple anæmia or with severe leukæmia absorb as much oxygen and excrete as much carbon dioxide as healthy men at rest and upon a similar diet.[7]

It was long ago shown by Spallanzani that living tissues removed from a recently killed animal took up oxygen and discharged carbon dioxide, and that this exchange was greater in most tissues than it was in blood. Similar experiments have been made by others.[8]

Paul Bert placed tissues from a recently killed dog in air for twenty-four hours, the temperature varying from about 0° to 10°, and obtained the following results:—

100 grms. of muscle		absorbed	50·8 c.c.	of oxygen, and discharged	56·8 c.c.	of carbon dioxide.
,,	brain	,,	45·8	,,	42·8	,,
,,	kidney	,,	37·0	,,	15·6	,,
,,	spleen	,,	27·3	,,	15·4	,,
,,	testis	,,	18·3	,,	27·5	,,
,,	broken bone & marrow	,,	17·2	,,	8·1	,,

[1] Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. x. S. 251; 1878, Bd. xviii. S. 247; 1893, Bd. liv. S. 333.

[2] Pflüger, *ibid.*, 1875, Bd. x. S. 251.

[3] Cohnheim, *Virchow's Archiv*, Bd. xlv.

[4] Oertmann, *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xv. S. 381.

[5] *Ibid.*, 1875, Bd. x. S. 368.

[6] Pembrey and Gürber, *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 449.

[7] Hannover, "De quantitate relativa et absoluta acidi carbonici ab homine sano et ægroto exhalati"; Abstract given by Möller, *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 546; Pettenkofer and Voit, *Ztschr. f. Biol.*, München, 1869, Bd. v. S. 319.

[8] Spallanzani, "Mém. sur la respiration," trad. par Senebier, 1803, p. 86; G. Liebig, *Arch. f. Anat., Physiol. u. wissensch, Med.*, 1850, Bd. xvii. S. 393; Matteucci, *Compt. rend. Acad. d. sc.*, Paris, 1856, tome xlii. p. 648; *Ann. de chim. et phys.*, Sér. 3, Paris, tome xlvii. p. 129; Valentin, *Arch. f. physiol. Heilk.*, Stuttgart, 1855, Bd. xiv. S. 431; 1857, N.F. Bd. i. S. 285; Bernard, "Leçons sur les propriétés physiol. des liquides," Paris, 1859, tome i. p. 403; Paul Bert, "Leçons sur la physiologie comparée de la respiration," Paris, 1870, p. 46; Regnard, "Rech. expér. sur les combustions respiratoires," Paris, 1879, p. 23.

In pure oxygen the tissues absorb more oxygen, but do not discharge a much greater quantity of carbon dioxide than they do in air; even in nitrogen or hydrogen the tissues continue to give off carbon dioxide.[1] The excised tissues of warm-blooded animals have a larger respiratory exchange than the corresponding tissues of cold-blooded animals, and differences are also observed in tissues from animals of different species.[2] The respiratory exchange of isolated muscle rises and falls, within certain limits, with the external temperature.[3]

Experiments made upon excised tissues are liable to several sources of error. Putrefaction may begin, and cause an absorption of oxygen and a discharge of carbon dioxide;[4] this danger, however, is small in tissues removed directly after the death of the animal, and kept at a low temperature, and free from septic contamination.[5] Another source of error is the loss of vitality in the tissues, and the accumulation of carbon dioxide and other waste products in the interior of the tissues.

A much better method for the study of the respiratory changes in isolated tissues and organs is that introduced by Ludwig;[6] an artificial circulation of blood is maintained, and the changes in the blood are determined. By these and similar experiments it can be shown that the tissues have the power of taking up oxygen, and also of oxidising various substances. This power is possessed in a different degree by the various tissues.[7] Schmiedeberg has shown that benzyl alcohol ($C_6H_5CH_2OH$), and the aldehyde of salicylic acid ($C_6H_4\begin{smallmatrix}COH\\OH\end{smallmatrix}$) undergo no appreciable oxidation when placed in blood, but if blood containing one of these substances is made to circulate through a freshly excised kidney then considerable quantities of benzoic acid ($C_6H_5.CO_2H$), or of salicylic acid ($C_6H_4\begin{smallmatrix}CO_2H\\OH\end{smallmatrix}$), as the case may be, are produced.

Ehrlich[8] found that most tissues could reduce and decolorise alizarine-blue and other pigments, but that the colour returned when the tissues were exposed to the air. Tissues placed in normal saline solution containing oxyhæmoglobin quickly reduce that substance, and in this respect muscle is the most effective. Bernstein[9] found the following values for the rate of reduction: Muscle 100, liver 81·47, involuntary muscle 72·4, and the mucous membrane of the stomach 57·05; lung tissue, on the other hand, had a very feeble power of reduction. This relative power of reduction holds good for tissues taken from frogs and from mammals. Somewhat similar experiments had been previously made by Yeo;[10] he supplied a frog's heart with

[1] Spallanzani, "Rapports de l'air avec les êtres organisés," par Senebier, Genève, 1807, tome i. p. 447; tome ii. pp. 44, 56.

[2] Paul Bert, *loc. cit.*

[3] Regnard, "Rech. expér. sur les combustions respiratoires," Paris, 1879, p. 23. See also "Animal Heat," this Text-book, vol. i. p. 840.

[4] Hermann, "Untersuch. u. d. Stoffwechsel der Muskeln," Berlin, 1867, S. 37.

[5] Tissot, *Arch. de physiol. norm. et path.*, Paris, 1894, tome xxvi. p. 838; 1895, tome xxvii.

[6] *Arb. a. d. physiol. Anst. zu Leipzig*, 1868.

[7] Schmiedeberg, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1876, Bd. vi. S. 233; 1881, Bd. xiv. S. 288, 379. For further details and references, see Neumeister, "Lehrbuch der physiol. Chemie," Jena, 1893, Th. 1, S. 8, *et seq.*

[8] "Der Sauerstoffbedürfniss des Organismus," Berlin, 1885.

[9] *Untersuch. a. d. physiol. Inst. d. Univ. Halle*, 1888, Heft 1, S. 107.

[10] *Journ. Physiol.*, Cambridge and London, vol. vi. p. 93. See also Vierordt, *Ztschr. f. Biol.*, München, 1875, Bd. xi. S. 195; Denning, *ibid.*, 1883, Bd. xix. S. 483.

solutions of fresh blood, and determined the reduction of the oxy-hæmoglobin by means of the spectroscope. The results show that the heart during contraction reduces the solution about ten times as quickly as when it is at rest.

The causes of the exchange of gases between the blood and the tissues.—The cause of the passage of oxygen from the blood to the tissues, and of carbon dioxide from the tissues to the blood, appears to be the difference in the tension of these gases in the tissues, and in the lymph and blood which surround them. It has been shown that the tissues have a great affinity for oxygen, and even store it up for the future oxidation of some of their constituents; and, on the other hand, that they are constantly producing carbon dioxide, and can even do this for a time in the absence of free oxygen.

The above conclusion is supported by the analyses of the gases of lymph and other secretions, and the determinations of the tensions of the gases in those fluids. Hammarsten[1] found that the lymph of a dog contained 0·1 volume per cent. of oxygen, 37·5 of carbon dioxide, and 1·6 of nitrogen. These results have been confirmed and extended by other observers.[2] Oxygen is present only in traces, but the quantity of carbon dioxide is less than that found in venous blood. This latter fact does not prevent the passage of carbon dioxide from the lymph to the venous blood, for Gaule[3] has shown that the tension of the gas is higher in the former fluid. It must be admitted, however, that further experiments are needed upon this point, for Gaule's experiments are not conclusive, and Strassburg found the tension of carbon dioxide in lymph to be intermediate between that in arterial and venous blood. Another probable cause of the smaller quantity of carbon dioxide in lymph is that many of the analyses were made upon lymph from the thoracic duct; the lymph would have been exposed in that situation to the action of arterial blood. This difficulty, however, is not present in some of the secretions. Thus, Strassburg[4] found in the urine and bile of a dog a tension of carbon dioxide equal to 9—7 per cent. of an atmosphere. Further, this physiologist has shown that, if air is injected into a ligatured portion of the intestine of a living dog, and after a short time is analysed, the tension of carbon dioxide is 7·7 per cent. of an atmosphere; that is, considerably greater than the tension of the gas in the venous blood.

These results are confirmed by the analyses[5] of some of the secretions of the body, and of various pathological transudations (see tables on p. 784).

Ewald also determined the tension of carbon dioxide in some of these fluids, and found results as high as 7·51, 10·92, 10·73, and 11·5 per cent. of an atmosphere. It is therefore permissible to conclude that the tension of carbon dioxide in the tissues which produce, and are in contact with, these fluids is higher than the tension of that gas in the venous blood.

[1] *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Cl.*, Leipzig, 1871, Bd. xxiii. S. 617.

[2] Daehnhardt and Hensen, *Virchow's Archiv*, Bd. xxxvii. S. 55, 68; Tschiriew, *Ber. d. k. sächs. Gesellsch. d. Wissensch. Math.-phys. Cl.*, Leipzig, 1874, Bd. xxvi. S. 120; Buchner, *Arb. a. d. physiol. Anst. zu Leipzig*, 1876, Bd. xi. S. 108.

[3] *Arch. f. Physiol.*, Leipzig, 1878, S. 469.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 94.

[5] Tables given by Halliburton, "Text-Book of Chemical Physiology and Pathology," London, 1891, p. 392.

	Dog.			
	Tissues.	Venous Blood.	Air of Alveoli.	External Air.
Tension of carbon dioxide in percentage of an atmosphere [1] . .	5 – 9	> 3·81 – 5·4	> 2·8	> 0·03

Secretion.	Oxygen.	Carbon Dioxide.			Nitrogen.	Observer.
		Removable by Vacuum.	Removable by Acid.	Total.		
	Vols. per cent.	Vols. per cent.	Vols. per cent.	Vols. per cent.	Vols. per. cent.	
Bile	0·2	14·4	41·7	56·1	0·4	Pflüger.[2]
,, ...	...	19·5	37·0	56·5	...	Bogoljubow.[3]
,, ...	...	17·1	62·5	79·6	...	,,
Submaxillary saliva (dog)	0·4	19·3	29·9	49·2	0·7	Pflüger.[4]
Submaxillary saliva (dog)	0·6	22·5	42·2	64·7	0·8	,,
Parotid saliva (human)	1·0	3–5	40–60	43·5–63·5	2·5	Kulz.[5]

Fluid.	Oxygen.	Carbon Dioxide.			Nitrogen.	Observer.
		Removable by Vacuum.	Removable by Acid.	Total.		
	Vols. per cent.	Vols. per cent.	Vols. per cent.	Vols. per cent.	Vols. per cent.	
Peritoneal . .	0·139	9·404	4·866	14·27	2·107	Planer.[6]
Hydrocele . .	0·16	32·49	32·45	64·94	2·05	Strassburg.[7]
Subcutaneous œdema	Traces	22·25	9·11	31·36	Traces	Ewald.[8]
Subcutaneous œdema (nephritis)	,,	21·88	31·18	53·06	,,	,,
Pleuritic . . .	0·68	39·34	15·59	54·93	1·33	,,
,, . . .	0·54	18·54	25·99	44·53	1·87	,,
,, . . .	...	18·64	41·16	59·80	...	,,
,, . . .	0·17	25·47	46·82	72·29	1·04	,,
Hydrothorax . .	0·29	25·34	48·67	74·01	0·87	,,
,, . .	1·01	25·71	55·50	81·21	2·47	,,

[1] Fredericq et Nuel, "Eléments de physiologie humaine," 1893, 3e édition, p. 158.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 173.
[3] *Centralbl. f. d. med. Wissensch.*, Berlin, 1869, No. 42; Kowalewsky and Arnstein, *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. viii. S. 598.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1868, Bd. i. S. 686.
[5] *Ztschr. f. Biol.*, München, 1887, Bd. xxiii. S. 321.
[6] *Ztschr. d. k.-k. Gesellsch. d. Aertze zu Wien*, 1859, No. 30.
[7] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. vi. S. 94.
[8] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1873, S. 663; 1876, S. 422.

ANIMAL HEAT.

By M. S. Pembrey.

Contents :—Thermometry, p. 785—Warm and Cold Blooded Animals, p. 787—Temperature of Man and other Warm-Blooded Animals, p. 788—Of Cold-Blooded Animals, p. 792—Hibernation, p. 794—Influence of Various Conditions upon Temperature, p. 798—Time of Day, p. 798—Age, p. 803—Muscular Work, p. 806—Mental Work, p. 807—Food, p. 809—Sleep, p. 810—Sex, p. 810—Race, p. 811—Menstruation and Pregnancy, p. 812—Individual Peculiarities, p. 812—Temperature of Surroundings, p. 812—Extreme Heat and Cold, p. 814—Baths, p. 818—Drugs, p. 820—Temperature of Different Parts of Body, p. 824—Of Arterial and Venous Blood, p. 826—Of the Skin, p. 829—Regulation of Temperature, p. 831—Heat Production, p. 832—Historical, p. 832—Relation to Chemical Changes, p. 833—Specific Heat of Body, p. 838—Seats of Heat Production, p. 839—Measurement of Heat Production, p. 844—Calorimetry, p. 844—Respiratory Exchange as a Measure of Heat Production, p. 847—Heat Production in Cold-Blooded Animals, p. 849—Regulation of Heat Loss, p. 850—Influence of Size of Body, p. 852—Influence of Nervous System, p. 854—Development of Power of Regulation, p. 865—Temperature of Body after Death, p. 866.

The higher animals have within their bodies some source of heat and some mechanism to regulate the production and loss of heat, for in the height of summer and in the depth of winter their mean temperature is constant. Of this fact the ancients had but an imperfect knowledge; they had no thermometers, and therefore could only judge from their sensations. Observations dependent upon the sensations of heat and cold are necessarily imperfect and often fallacious. The invention, therefore, of thermometers was imperative, if exact data upon the temperature of animals were to be obtained

The Introduction of Thermometers.—Towards the close of the sixteenth century the first thermometers appear to have been made.[1] The credit of the invention has been attributed chiefly to Sanctorius of Padua, and Galileo; the former based his thermometer upon the expansion of air enclosed in a bulb at the end of a tube which contained a coloured liquid; while Galileo is said to have made, in 1612, the first alcohol thermometer. Boyle introduced the alcohol thermometer into England, where Hooke, in 1665, recommended that the zero of the scale should be the freezing point of water, which he and Boyle found to be constant. In 1680, Newton suggested the boiling point of water for a further graduation of the thermometer, and Halley a few years later proved that the point was a constant one, and recommended the use of mercury in the construction of thermometers. Fahrenheit first replaced spirit by mercury in 1720, and, after several attempts at graduation, introduced the scale which now bears his name. The introduction of the centigrade ther-

[1] Holloway, "The Evolution of the Thermometer," *Sc. Prog.*, London, 1895–96, vol. iv. p. 413; Liebermeister, "Handbuch d. Path. u. Therap. des Fiebers," Leipzig, 1875, S. 3.

mometer was due to Celsius in 1742.[1] Throughout this article the centigrade scale is employed.

The Determination of Temperature in Different Parts of the Body.

Varying quantities of heat are produced and lost in different parts of the body, and although the circulation of the blood tends to bring about a mean temperature of the internal parts, local differences are present. It is important, therefore, that the determinations should be made in those parts which have a temperature representing the internal temperature; and in order that the results may be comparable, the observations should as far as possible be made in similar anatomical positions.

The most suitable place for the application of the thermometer varies under different conditions, and methods have to be considered, not only in as far as they are scientifically sound, but also in respect to their ease in practice.

The *rectum* naturally offers the readiest access to the internal parts, and thermometers with or without a metal guard may be safely introduced 5 or 6 cms. This method is the most suitable in the case of animals, and may be advantageously employed in infants. The *vagina*, *uterus*, and *bladder* of women and female animals of suitable size have a similar value to that of the rectum.

In order to obtain the internal temperature of the body, the bulb of the thermometer, previously warmed in the mouth, may be inserted in the stream of *urine* as it leaves the urethra.[2] Apart, however, from the limited applicability of this method, there is a danger of a loss of heat by evaporation and radiation, but with care excellent results may be obtained.

The *axilla* is a convenient place for thermometric determinations in man, for it is not liable to great variations in temperature. It is necessary, however, that the axilla be closed well and long enough for it to attain the temperature of a closed cavity; in very thin or wasted subjects it is difficult to effect this, and the temperature should therefore be taken elsewhere in such cases.

The *groin* has also been selected by some physicians for the observation of temperature, but in man it is not so easy to retain the thermometer in the fold of the groin as in the closed axilla. The method is useful in the case of infants.

The *mouth*, on account of convenience, has been widely selected for the clinical observation of temperature, but the readings of a thermometer, even when the bulb is placed under the tongue and the mouth is firmly closed, are liable to be low, owing to the danger of cooling of the tissues of the mouth, externally by cold air, internally by the inspired air. The mouth is also liable to considerable local variation of temperature.

In order to obtain accurate results, the thermometer should be retained for eight minutes in the mouth, ten minutes in the well-closed and dry axilla, and

[1] For an account of the introduction of the thermometer into clinical use, see Wunderlich, "Medical Thermometry," *New Syd. Soc. Translation*, 1871, p. 19; Lorain, "De la temperature du corps humain," Paris, 1877, tome i. p. 39 *et seq.*

[2] Stephen Hales, "Statical Essays," London, 1731, 2nd edition, vol. i. p. 59; Martine, "Essays, Medical and Philosophical," 1740, p. 335; Blagden, *Phil. Trans.*, London, 1775, vol. lxv. pt. 1, p. 114; Davy, *ibid.*, 1844, pt. 1, p. 63; Mantegazza, *Presse méd. belge*, Bruxelles, 1863, tome xv. p. 111; Oertmann, *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvi. S. 101.

for three or four minutes in the rectum or vagina;[1] it should be kept in position for a minute or two after the mercury has become stationary.

Different values have been given by various observers for the temperature in the mouth, axilla, and rectum; these will be critically examined later, but at present it may be stated that the temperature in the rectum is generally about three- or four-tenths higher than that in the axilla or mouth. Under certain circumstances, however, this relationship is altered. Thus Bosanquet[2] found that, although the temperature in the rectum was almost invariably higher than that in the mouth, the average difference being four-tenths, yet on some occasions, as immediately after eating, the temperature in the mouth exceeded that in the bowel; while on others, as during vigorous exercise, the heat of the mouth sank considerably, *e.g.* to 35°·6 (96° F.), that of the rectum rising to 37°·7 (99°·8) or 37°·8 (100°). Violent exercise was found by Davy[3] to lower the temperature in the mouth, and raise that in the axilla. The probable explanations of these differences are that vigorous exercise would, by the increase of respiration, cool the mouth, and by increasing the vascularity of the axilla raise the heat in that part. The increase in the temperature of the mouth immediately after eating is probably due to the increase in the blood supply and activity of the muscles and glands in that cavity.

In order to obtain the maximal temperature of the interior of the body, Kronecker and Meyer[4] used small bulbs of mercury, made according to the principle of Dulong and Petit's outflow thermometer. The animal was made to swallow the small bulb, which, after evacuation by the bowel, was placed in water gradually warmed until the mercury expanded to the point of outflow; the temperature of the water represented the maximal temperature of the body. It was found by this method that the maximal temperature of a dog was 39°·2 and that of a rabbit 40°·2, the rectal temperatures varying respectively from 37°·8 to 38°·2, and 37°·0 to 37°·9. Special thermo-electric methods will be mentioned in the discussion of surface temperature.

Warm-blooded and cold-blooded animals.—An important difference in temperature exists between the higher and lower animals. Those animals which are high in the scale of evolution, such as birds and mammals, have a high temperature, which is fairly constant and independent of the temperature of the surrounding air. The lower animals, on the contrary, have a temperature dependent upon, and only slightly above, that of their surroundings, and thus liable to considerable variations. This difference between the two classes is expressed by the terms "warm-blooded" and "cold-blooded" animals. The classification, however, is not absolutely exact, for there are mammals, such as the marmot, hedgehog, bat, and dormouse, which are in an intermediate position; in warm weather these animals have a high temperature, which is fairly constant and independent of their surroundings, but in winter they become inactive, they hibernate, and their temperature falls and varies with that of their surroundings. On the other hand, there are bees, animals of a much lower order, which have and maintain

[1] Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. pp. 554–559.
[2] *Lancet*, London, 1895, vol. i. p. 672.
[3] "Researches," London, 1839, vol. i. p. 199.
[4] *Arch. f. Physiol.*, Leipzig, 1878, S. 546.

a higher temperature than that of most cold-blooded animals, and are not reduced to spend the winter in a torpid state.

Even in the case of the most perfectly warm-blooded animals there is a stage in which they resemble cold-blooded animals; infants and young animals born in an immature condition cannot maintain the temperature of their bodies at the normal height of the temperature of the adult; they need some accessory source of heat, such as the warmth of the parent's body.

The terms "warm-blooded" and "cold-blooded" are inexact, for the temperature of a so-called cold-blooded animal living in the tropics may, under some circumstances, equal that of a mammal. John Hunter[1] showed that the essential difference in the two classes was in the constancy and inconstancy of the temperature of the two groups, and he suggested that the warm-blooded animals should be called "animals of a permanent heat in all atmospheres;" the cold-blooded, "animals of a heat variable with every atmosphere." Again, in 1845, Donders[2] pointed out the same fact, and called the two groups of animals, those with a constant and those with an inconstant temperature. A year or two later, Bergmann[3] discussed very fully the objections to the old terms, and suggested the definitions, "animals with a constant temperature and animals with a varying temperature, or *homoiothermic* and *poikilothermic* animals." In the present article, however, the terms "warm-blooded" and "cold-blooded" are retained, for they have been sanctioned by long usage, and their meaning is well understood. A further reason for their retention is found in the fact that there is no hard-and-fast line between the animals with a constant temperature and those with a varying temperature.

The temperature of man and other warm-blooded animals.—*The temperature of man.*—The mean daily temperature of a healthy man varies slightly according to the part of the body in which it is observed: in the rectum it is 37°·2 (98°·96 F.), in the axilla 36°·9 (98°·45 F.), in the mouth 36°·87 (98°·36 F.). These figures are the averages selected from the different observations given in the table on p. 789, and represent the mean temperature of a working day.

The normal temperature of man is generally stated, as the result of John Davy's numerous observations, to be 36°·9 (98°·4 F.) in the mouth. This, however, is wrongly looked upon as the mean temperature of twenty-four hours, for it represents the mean of observations taken chiefly during the active part of a day, from about 8 A.M. to 12 o'clock midnight; all observers agree that the lowest temperatures are found between midnight and early morning, and for very evident reasons the observations during this period are few. The mean temperature of twenty-four hours is therefore without doubt below 36°·9 (98°·4 F.), and the observations of Casey, Clifford Allbutt, and Ogle show that this figure is even too high for the mean temperature of a working day. The average obtained from their results is 36°·7 (98°·14 F.) for the temperature taken in the mouth. The observations upon the temperature between midnight and morning are so few, that it is impossible at present to give the mean temperature of a day of twenty-four hours.

[1] "Works," Palmer's edition, London, 1837, vol. iii. p. 16.

[2] "Der Stoffwechsel als die Quelle der Eigenwärme bei Pflanzen und Thieren," Wiesbaden, 1847, S. 12-13.

[3] "Göttinger Studien," 1847, Abth. 1, S. 595.

Maximum.	Minimum.	Mean Daily Temperature.	Place of Observation.	Observer.
37°·2	36°·5	36°·9	Mouth	Davy.[1]
37°·5	36°·8	37°·2	,,	Gierse.[2]
37°·0	36°·3	36°·7	,,	Hooper.[3]
37°·36	36°·63	37°·05	,,	Hallmann.[4]
37°·14	36°·63	36°·93	,,	Lichtenfels and Fröhlich.[5]
37°·12	36°·39	36°·81		
37°·0	36°·1	36°·7	,,	Casey.[6]
37°·0	36°·6	36°·8	,,	Clifford Allbutt.[7]
37°·0	36°·2	36°·65	,,	Ogle.[8]
38°·0	36°·2	37°·0	Axilla	Wunderlich.[9]
37°·3	36°·1	...	,,	Ringer and Stuart.[10]
37°·44	36°·15	36°·89	,,	Liebermeister.[11]
37°·13	36°·73	36°·9	,,	Damrosch.[12]
37°·4	36°·1	36°·7	,,	Billet.[13]
37°·9	36°·3	37°·1	,,	Billroth.[14]
37°·8	36°·5	37°·2	Rectum	Jürgensen.[15]
37°·1	36°·6	36°·85	,,	Neuhauss.[16]
...	...	37°·1	,,	Bosanquet.[17]
37°·35	36°·95	37°·13	,,	Jaeger.[18]
37°·4	36°·15	36°·8	,,	Nicol.[19]
37°·3	36°·1	36°·9	Urine	Richet.[20]
37°·95	36°·4	37°·2	,,	Mantegazza.[21]
...	...	36°·9	,,	Gley.[22]
...	...	37°·1	,,	Rondeau.[23]
37°·6	36°·2	36°·9	,,	Pembrey.[24]

The temperature of other warm-blooded animals.—The observations upon the temperature of animals are numerous, but have not been repeated often enough under different conditions which are known to affect the temperature of man. On this account, and also because animals are known to have a somewhat variable temperature, it is impossible in most cases to give the mean temperature. The following table gives some of the results obtained by different observers:—

[1] *Phil. Trans.*, London, 1845, pt. 2, p. 319.
[2] "Quænam sit Ratio Caloris Organici, etc.," Halae, 1842, p. 40.
[3] *Med. Times and Gaz.*, London, 1866, vol. ii. p. 483.
[4] Quoted from Landois, "Lehrbuch d. Physiol.," Aufl. 3, S. 406.
[5] *Denkschriften d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1852, Bd. iii. Abth. 2, S. 113.
[6] *Lancet*, London, 1873, vol. i. p. 200.
[7] *Journ. Anat. and Physiol.*, London, 1872, vol. vii. p. 106.
[8] *St. George's Hosp. Rep.*, London, 1866, vol. i. p. 221.
[9] "Medical Thermometry," p. 95.
[10] *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 186.
[11] "Handbuch d. Path. u. Therap. d. Fiebers," 1875, S. 78.
[12] *Deutsche Klinik*, Berlin, 1853, Bd. v. S. 317.
[13] Thèse, Strasbourg, 1869.
[14] *Arch. f. klin. Chir.*, Berlin, 1862, Bd. ii. S. 331.
[15] "Die Körperwärme des gesunden Menschen," Leipzig, 1873.
[16] See p. 813.
[17] *Lancet*, London, 1895, vol. i. p. 672.
[18] *Deutsches Arch. f. klin. Med.*, Leipzig, 1881, Bd. xxix. S. 522.
[19] Result of observations not yet published.
[20] *Rev. scient.*, Paris, 1885, tome ix. p. 629.
[21] *Presse méd. belge*, Bruxelles, 1863, tome xv. p. 111.
[22] Quoted from Richet, *Rev. scient.*, Paris, 1885, tome ix. p. 432.
[23] *Ibid.*
[24] Result of observations not yet published.

Animals.	Average Rectal Temperature.	Extremes of Observations.	Number of Observations.	Observer.
Horse	37°·9 (100°·2)	37°·2–38°·6	On 150 horses	Strecker.[1]
	37°·7 (99°·9)		600 on 100 ,,	Föhringer.[1]
	37°·9 (100°·2)	36°·1–38°·6	On 212 ,,	Hobday.[2]
Ox	38°·85 (101°·9)	37°·7–40°·3	On 352 cows and oxen	Robertson.[3]
Cow	38°·9 (102°·0)	38°·7–39°·1	39 on 1 cow	Siedamgrotzky.[4]
	38°·6 (101°·5)	37°·5–39°·4	On 87 cows	Hobday.[2]
	38°·6 (101°·5)	37°·7–39°·6	On 100 cows	Singleton.[5]
Sheep	40°·6 (105°·1)	40°·0–41°·07	On 24 sheep	Davy.[6]
	40°·2 (104°·4)	38°·5–41°·8	284 on 6 ,,	Siedamgrotzky.[4]
	40°·0 (104°·0)	39°·7–40°·2	On more than 100 sheep	Hobday.[2]
	40°·1 (104°·2)	39°·6–41°·0	On 100 sheep	Singleton.[5]
Dog	38°·3 (100°·9)	...	190 on 17 dogs	Siedamgrotzky.[4]
	37°·91 (100°·2)	37°·15–38°·45	44 on several ,,	Hoppe.[7]
	38°·8 (101°·8)	38°·3–39°·9	6 on 6 ,,	Obernier.[8]
	38°·6 (101°·5)	38°·1–39°·2	On more than 200 dogs	Hobday.[2]
	38°·8 (101°·8)	38°·0–39°·8	On 100 dogs	Singleton.[5]
Cat	38°·7 (101°·7)	37°·9–39°·7	On 41 cats	Hobday.[2]
Pig	39°·6 (103°·3)	38°·3–40°·8	169 on 4 young pigs	Siedamgrotzky.[4]
	38°·7 (101°·7)	38°·7–39°·3	On more than 100 pigs	Hobday.[2]
Rabbit	39°·2 (102°·5)	37°·3–39°·9	72 on 27 rabbits	Hale White.[9]
	38°·8 (101°·8)	38°·0–39°·5	7 on 7 ,,	Obernier.[8]
	38°·7 (101°·7)	37°·0–40°·8	31 on 10 ,,	Pembrey.
Ferret	39°·3 (102°·8)	37°·9–40°·4	On 8 ferrets	Hobday.[2]
Guinea-pig	38°·7 (101°·7)	38°·5–39°·4	About 50 observations	Finkler.[10]
	37°·93 (100°·2)	37°·0–39°·2	19 on 5 guinea-pigs	Pembrey.
	39°·21 (102°·6)	37°·9–40°·2	35 observations	Richet.[11]
	37°·4 (99°·4)	36°·0–38°·5	40 on 4 guinea-pigs	Colasanti.[12]
	38°·85 (101°·9)	38°·0–39°·6	30 on 1 guinea-pig	Pitts.
Rat (black and white)	37°·5 (99°·5)	37°·0–38°·5	16 on 4 rats	Pembrey.
Rat (white)	37°·96 (100°·3)	37°·4–38°·9	60 on 2 rats	Pitts.
Mouse (black and white)	37°·4 (99°·3)	36°·1–38°·6	27 on 8 mice	Pembrey.
Monkey (*Rhesus*)	38°·4 (101°·1)	36°·9–39°·7	22 on 2 monkeys	Hale White and Washbourn.[13]
Echidna (*Hystrix*)	27°·5 (81°·5) (cloaca or in abdomen)	25°·5–30°	5 on 2 specimens	Miklouchо Maclay.[14]
	32°·5 (90°·5) (cloaca)	26°·5–34°·2	7 on 7 specimens	Semon.[15]
Ornithorhynchus	24°·8 (76°·6) (cloaca)	24°·4–25°·2	2 on 1 specimen	Miklouchо Maclay.[14]

[*Continued on next page.*

[1] Ellenberger, "Vergleichende Physiol. der Haussäugethiere," 1892, Bd. ii. Th. 2, S. 81.
[2] *Journ. Comp. Path. and Therap.*, Edin. and London, 1896, vol. ix. p. 286.
[3] *Veterinary Journ.*, London, 1885, vol. xx. p. 311.
[4] *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1875, Bd. i. S. 87.
[5] *Veterinarian*, London, 1888.
[6] "Researches," London, 1839, vol. i. p. 208.
[7] *Virchow's Archiv*, 1857, Bd. xi. S. 459.
[8] "Der Hitzschlag," Bonn, 1867.
[9] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 1.
[10] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxix. S. 112.
[11] *Rev. scient.*, Paris, 1884, tome viii. p. 306.
[12] *Arch. f. d. ges. Physiol.*, Bonn, 1877, Bd. xiv. S. 123.
[13] *Journ. Anat. and Physiol.*, London, vol. xxv. p. 379.
[14] *Proc. Linn. Soc. New South Wales*, 1883, vol. viii. p. 425 ; vol. ix. p. 1205.
[15] *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 229.

Animals.	Average Rectal Temperature.	Extremes of Observations.	Number of Observations.	Observer.
Fowl (common)	42°·8 (109°·0)	41°·7–43°·9	On 14 fowls	Davy.[1]
	41°·6 (106°·9)	40°·6–43°·0	On 111 fowls	Hobday.[2]
Duck	43°·6 (110°·5)	43°·4–43°·9	On 8 ducks	Davy.[1]
	42°·1 (107°·8)	41°·4–43°·0	On 24 ducks	Hobday.[2]
Goose	41°·7 (107°·0)	41°·1–41°·7	On 5 geese	Davy.[1]
Pigeon	40°·9 (105°·6)	40°·0–42°·5	20 on 4 pigeons	Corin and Van Beneden.[3]
Ostrich	37°·3 (99°·2)	36°·9–37°·8	On 5 ostriches	Hobday.[2]

In the next table are collected the results of various observations upon other mammals and birds; in most of these cases the figure given for the temperature represents the result of a single observation[4]:—

Animal.	Temperature.	Place of Observation.	Observer.
Monkey (*Simia aygula*)	39°·7 (103°·5)	Rectum	Davy.
Ass	36°·95 (98°·5)	,,	Hunter.[5]
Elk	39°·4 (103°)	,,	Davy.[6]
Goat	39°·4 (103°)	,,	,,
Tiger	37°·2 (99°)	,,	,,
Ichneumon	39°·4 (103°)	,,	,,
Squirrel	38°·9 (102°)	,,	,,
Manatee	39°·4 (103°)	Abdomen	Martine.[7]
Whale	38°·8 (101°·8)	,,	,,
Greenland whale	38°·9 (102°)	,,	Scoresby.[8]
Seal	38°·9 (102°)	,,	Tiedemann.[9]
Porpoise	37°·5 (99°·5)	,,	,,
Pigeon	42°·2 (108°)	Rectum	Davy.
Thrush	42°·8 (109°)	,,	,,
Turkey	42°·8 (109°)	,,	,,
Guinea-fowl	43°·3 (110°)	,,	,,
Pheasant	42°·6 (108°·7)	,,	Richet.[10]
Great titmouse	44°·0 (111°·2)	,,	Tiedemann.
Sparrow	42°·1 (107°·8)	,,	Davy.
Swift	44°·0 (111°·2)	,,	Tiedemann.
Heron	41°·0 (105°·8)	,,	Prévost and Dumas.[11]
Redwing	43°·3 (109°·9)	,,	Hobday.
Fieldfare	43°·7 (110°·6)	,,	,,
Yellowhammer	43°·2 (109°·8)	,,	,,

The above tables show that the rectal temperature of most of the mammals is higher than that of man; the most marked exception is found in the monotremata, the lowest group of the mammalia; thus the temperature of the porcupine echidna (*Echidna hystrix*) varies from 25°·5 to 34°·2, that of the duckbilled platypus (*Ornitho-*

[1] "Researches," London, 1839, vol. i. p. 186.
[2] *Journ. Comp. Path. and Therap.*, Edin. and London, 1896, vol. ix. p. 286.
[3] *Arch. de biol.*, Gand, 1887, tome vii. p. 265.
[4] For the temperature of other animals, see Gavarret's "De la chaleur produite par les êtres vivants," Paris, 1855, p. 92; Richet, *Rev. scient.*, Paris, 1884, tome viii. p. 298.
[5] "Works," Palmer's edition, vol. iii. p. 340.
[6] "Researches," London, 1839, vol. i. pp. 181, 188.
[7] "Essays, Medical and Philosophical," 1740, p. 337.
[8] Milne Edwards, "Leçons," tome viii. p. 16.
[9] "Physiologie," Bd. i. S. 454.
[10] *Rev. scient.*, Paris, 1885, tome ix. p. 202.
[11] *Ann. de chim. et phys.*, Paris, Sér. 2, tome xxiii. p. 61.

rhynchus anatinus) from 24°·4 to 25°·2. In the case of birds the temperature is generally two or three degrees higher than that of mammals.

In the observation of the temperature of animals, it is necessary, if comparable results are to be obtained, to insert the thermometer to a similar extent each time, and to prevent struggling of the animal before and during the time of observation. Finkler [1] found that the rectal temperature of guinea-pigs was 36°·1, 38°·7, and 38°·9, at a depth of 2·5, 6, and 9 cms. respectively. Aronsohn and Sachs [2] found that the rectal temperature of normal rabbits rose to over 40° after a short chase, Hobday [3] observed a rise to 41°·1 in the case of sheep and pigs, and Mott [4] has noticed a rise of one or two degrees in the temperature of monkeys, owing to a similar cause. Moreover, the times of observation should as far as possible be similar, for animals show a daily variation in temperature. Rabbits extended on their backs and tied down lose so much heat that their temperature rapidly falls (Legallois, Richet.[5])

The temperature of cold-blooded animals.—It has already been shown that there is no hard and fast line between the so-called warm-blooded animals—those with a constant temperature, and the cold-blooded animals—those with a varying temperature. Further proofs of this will now be given, and others will be brought forward when the subject of hibernation is considered.

John Hunter [6] made some interesting observations upon the temperature of bees. He found in the month of July, when the temperature of the air was 12°·2, and a north wind was blowing, that the temperature at the top of a hive full of bees was 27°·8. In December the temperature of the hive was 22°·8, when that of the external air was only 1°·7. A single bee has so little power of keeping itself warm, that it quickly becomes numb and almost motionless when exposed to the moderate cold of a summer night. The aggregation, however, of vast numbers in a hive ensures the production of enough heat to keep the bees active even in winter, and for this production of heat a constant supply of food is necessary. The warmth of the hive is needed also for the eggs, pupæ, and larvæ, for Hunter found that they would not live in a temperature of 17°. The wax is by means of the warmth kept so soft that the bees can model it with ease.

Numerous observations upon the temperature of bees were made by Newport,[7] who found that, when the insects were in a state of activity, their temperature was above that of their surroundings; the larva and pupa had a lower temperature than the imago, and less power of generating as well as of maintaining their temperature. In winter the temperature of a hive, when the bees were in a state of repose, fell considerably, and varied slowly with that of the atmosphere; the bees did not become torpid, but passed into a deep sleep, broken at intervals by periods of activity. A very low atmospheric temperature aroused the bees, and thus prevented any great fall in the temperature of the hive. Thus on January 2, 1836, at 7.15 A.M., when the temperature of the air was −7°·5, that of the hive was −1°·1, and the bees were quiet, but after the bees were disturbed by tapping the hive, the temperature

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1882, Bd. xxix. S. 117.
[2] *Ibid.*, 1885, Bd. xxxvii. S. 232.
[3] *Journ. Comp. Path. and Therap.*, Edin. and London, 1896, vol. ix. p. 286.
[4] Note communicated to the writer.
[5] *Rev. scient.*, Paris, 1884, tome viii. p. 300.
[6] "Works," Palmer's edition, London, 1837, vol. iv. p. 427.
[7] *Phil. Trans.*, London, 1837, pt. 2, p. 253.

was raised to 21°·1 within fifteen minutes. On another occasion, when the external temperature was 1°·4, that of the hive full of active bees was 38°·9. The temperatures of individual nurse bees, brooding over the young bees in the combs, was as high as 29°·4, while the temperature of the cell after the bee left it was 24° and that of the air 22°.

Similar results have been obtained by Reaumur,[1] Huber,[2] Dutrochet,[3] Nobili and Melloni,[4] and others.

In marked distinction to bees are other insects, such as some wasps and flies, which can pass the winter in a state of torpor, their temperature varying with that of their surroundings.

The difference between the temperature of the animal and that of its surroundings varies in different classes of the cold-blooded animals. The following are results obtained by different observers:—

Animal.	Temperature of Animal.	Temperature of Surroundings.	Observer.
Viper . . .	20° (68°)[5]	14°·4	Hunter.[6]
Python . . .	24°·4 (76°)	15°·6	Sclater.[7]
Turtle . . .	28°·9 (84°)[5]	26°·4	Davy.[8]
,, . . .	27°·8 (82°)	28°·9	Czermach.[9]
Frog . . .	17°·2 (63°)[5]	16°·7	Davy.[8]
,, . . .	14°·4 (58°)[5]	14°·4	,,
,, . . .	8°·9 (48°)	6°·7	Czermach.[9]
Proteus . . .	17°·8 (64°)	13°·3	,,
Carp . . .	20°·6 (69°)	18°·6	Hunter.[6]
Trout . . .	14°·4 (58°)	13°·3	Davy.[8]
,, . . .	5°·6 (42°)	4°·4	,,
Flying fish . .	25°·6 (78°)	25°·3	,,
Shark . . .	25° (77°)	23°·7	,,
Bonito . . .	37°·2 (99°)	26°·9	,,
Crayfish . .	26°·1 (79°)	26°·7	,,
Crab . . .	22°·2 (72°)	22°·2	,,
Snail (Indian) .	24°·6 (76°·25)	24°·6	,,
Earthworms . .	14°·7 (58°·5)	13°·3	Hunter.[6]
Black Slugs . .	13° (55°·25)	12°·2	,,
Leeches . .	13°·9 (57°)	13°·3	,,
Scarabæus . .	25° (77°)	24°·4	Davy.[8]
Glow-worm . .	23°·3 (74°)	22°·8	,,
Locust . . .	22°·2 (72°·5)	16°·7	,,
Papillio Agamemnon	27° (80°·5)	25°·6	,,
Scorpion . .	25°·3 (77°·5)	26°·1	,,

The results of observations on the temperature of other cold-blooded animals will be found recorded in the works of Tiedemann,[10] Rudolphi,[11] Newport,[12] Valentin,[13] Dutrochet,[14] Milne Edwards,[15] and Gavarret.[16]

[1] "Mém. pour servir à l'histoire des insectes," Mém. 13, tome iv.
[2] "Nov. obser. sur les abeilles," tome ii. p. 336.
[3] *Ann. d. sc. nat.*, Paris, 1840, "Zoologie," Sér. 2, tome xiii. p. 5.
[4] *Ann. d. chim. et phys.*, Paris, Sér. 2, tome xlviii. p. 207.
[5] Rectal.
[6] "Works," Palmer's edition, London, 1837, vol. iv. p. 131 *et seq.*
[7] *Proc. Zool. Soc.*, London, 1862, p. 365.
[8] "Researches," London, 1839, vol. i. p. 189; *ibid.*, p. 219.
[9] *Journ. de phys.*, Paris, 1821.
[10] "Physiologie," Bd. i. S. 454.
[11] "Grundriss der Physiol.," Bd. i. S. 151 *et seq.*
[12] *Phil. Trans.*, London, 1837, pt. 2, p. 259.
[13] *Repert. f. Anat. u. Physiol.*, 1839, Bd. iv. S. 359.
[14] *Ann. d. sc. nat.*, Paris, 1840, "Zoologie," Sér. 2, tome xiii. p. 5.
[15] "Leçons sur la physiol.," tome viii. p. 7.
[16] Article "Chaleur animale," "Dictionnaire encyclopédique d. sciences médicales," Paris, 1874, Sér. 1, tome xv.; "De chaleur produite par les êtres vivants," Paris, 1855, p. 113.

A consideration of the above lists shows that the temperature of cold-blooded animals is generally a few tenths of a degree above that of their surroundings, but that in some exceptional cases, as that of the python and a fish known as the bonito (*Thynnus pelamys*), it may be 10 degrees above the external temperature. Although these high temperatures are well authenticated, the causes have not been determined; it is to be noted, however, that the high temperature is more marked in the incubating female python than in the male which does not incubate, and that the bonito has very vascular red muscles.[1]

The temperature of many of the cold-blooded animals is often below that of the air, owing to the great loss of heat by evaporation, and to the large surface exposed, especially by insects, to cooling by radiation and conduction.

Hibernation.[2]—Certain animals, on the approach of winter, and in some cases even in summer, retire to their burrows or other shelter, become inactive, and fall into a torpid state. All the activities of the body are greatly reduced, and the temperature falls to a point only slightly above that of the surroundings. Such is the condition known as hibernation.

The animals in whom hibernation has been definitely proved to take place, do not belong to any one class; examples are met with in mammals, reptiles, amphibians, insects,[3] molluscs, and lower animals, but no cases are known among birds. As regards fishes, no well-authenticated cases of hibernation are known; there are doubtful instances in which the fish has been imprisoned by the freezing of the water, and yet has remained alive for some time.

The following mammals hibernate — spermophile, marmot, hamster, squirrel, hedgehog, dormouse, bat, bear, and beaver. In some cases the animal lays up stores of food, upon which it feeds when it awakes at intervals during the period of hibernation; in other cases, there is a special accumulation of fat within the animal's body before the commencement of the torpid state.

The further account of this subject refers only to the hibernating mammals.

The condition of the animal during hibernation. — Respiration. — The frequency of respiration is greatly diminished, and the rhythm is irregular and often of the Cheyne-Stokes type. A hibernating dormouse may not give a single respiration for ten minutes, then may take ten or fifteen breaths, and again cease breathing for another period of several minutes. The same animal in an active condition breathes at the rate of eighty or more in a minute. Similar results have been obtained in the case of other animals.

Determinations of the respiratory exchange have been made. Spallanzani[4] found that during hibernation marmots and bats could be kept for four hours in carbon dioxide gas without suffering any ill effects, whereas a bird and a rat placed in the chamber at the same time died at once. Saissy[5] observed that the amount of oxygen taken up by dormice varied as the activity of the animal, and that during well-marked hibernation there was hardly any intake.

[1] See p. 849.

[2] Since this section was written, there has appeared a monograph by Dubois, "Physiologie comparée de la marmotte," Paris, 1896, which contains a large number of original observations and an abstract of the previous work upon hibernation. The bibliographical index contains references to 145 papers.

[3] Trimen, "Butterflies of South Africa," vol. i. p. 231. See also *Nature*, London, 2nd April and 11th June 1896.

[4] Spallanzani, "Memoirs on Respiration," edited by Senebier, 1804. See article "Chemistry of Respiration," this Text-book, vol. i.

[5] "Recherches expérimentales anatomiques, chimiques," etc., 1808; Reeve, "On Torpidity," 1809; Edwards, "De l'influence des agens physiques sur la vie," Paris, 1824.

These results have been extended and confirmed by Marshall Hall,[1] Regnault and Reiset, Horvath,[2] and others.[3]

Regnault and Reiset[4] determined the respiratory exchange of several hibernating marmots, and found that the intake of oxygen was about one-thirtieth of that of an active animal, and only about two-fifths of the oxygen appeared in the carbon dioxide discharged. The following are two examples:—

Condition of Marmot.	Grms. per Kilo. and Hour.		
	O_2 Intake.	CO_2 Output.	$\frac{CO_2}{O_2}$
Hibernating .	0·48	0·37	·566
Awake . .	1·198	1·312	·796

A further proof that oxygen was stored up in the body of the hibernating animal was found in the increase in weight of a marmot during profound torpidity; it gained as much as 5·9 grms. in five days.

The output of carbon dioxide was investigated by Horvath, who found that the amount varied according to the animal's activity. The following is an example of his results:—

Animal.	Condition.	Rectal Temperature.	Temperature of Air.	Respirations per Minute.	CO_2 in Grms.
Sisel,[5] 163 grms.	Hibernating	9°	9°	5	·025 in three hours.
...	Awake	33°·5	13°	95	·457 in half an hour.

Similar results have been obtained in the case of dormice and bats.[6]

According to Saissy,[7] a hedgehog can absorb all the oxygen from the air in which it is confined, and can even live for fifteen minutes in pure nitrogen, whereas a rat under similar conditions dies in less than three minutes.

Circulation.—The force and frequency of the heart-beat is much reduced during hibernation; in the case of the bat and dormouse to fourteen and sixteen per minute or even less, the rate in the active animal being above 100 per minute. On applying a stethoscope to the chest of a hibernating bat, no sound of the heart-beat can be heard, whereas, when the animal awakes and becomes active, the sounds are so loud that they can be heard by the ear placed one inch away from the animal (Hill and Pembrey).

[1] *Phil. Trans.*, London, 1832, pt. 2, p. 335; Barkow, "Der Winterschlaf," 1846, here numerous additional references are given.

[2] *Verhandl. d. phys.-med. Gesellsch. in Wurzburg*, 1878, Bd. xii.; 1879, Bd. xiii.; 1880, Bd. xiv.; 1881, Bd. xv.

[3] Pembrey and Hale White, *Journ. Physiol.*, Cambridge and London, 1895–96, vol. xix. p. 477.

[4] *Ann. d. chim. et phys.*, Paris, 1849, Sér. 3, tome xxvi. p. 429.

[5] Allied to the marmots.

[6] Pembrey and Hale White, *loc. cit.*

[7] *Deutsches Arch. f. d. Physiol.*, Halle, 1817, Bd. iii. S. 135.

The blood during hibernation has, according to most observers,[1] an arterial colour in the veins; on the other hand, Marshall Hall states that it has a venous hue even in the arteries. Further details concerning the circulation will be found in the works of Reeve, Edwards, Barkow, Horvath, and Dubois.[2]

The gases in the blood of hibernating and of active marmots have been determined by Dubois,[3] who found that during hibernation the arterial blood contained as much oxygen, the venous blood less oxygen, and both arterial and venous blood an excessive quantity of carbon dioxide, as compared with the gases of arterial and venous blood from active animals.

Digestion.—The activity of the digestive organs varies according to the habits of the different animals; some, such as bats, take no food during the winter; others, such as the dormouse, hamster, and marmot, store up food, which they consume during short periods of activity.[4]

Nervous system.—The excitability of the nervous system is greatly depressed, and the nervous and other tissues of the body resemble those of cold-blooded animals, in retaining their excitability for a long time after removal from the body.[4]

Temperature.—During hibernation the temperature resembles that of a cold-blooded animal, rising and falling with that of the surroundings. In this way the rectal temperature may fall as low as 2° without injurious effects following. When the animal awakes from hibernation its temperature generally rises rapidly many degrees above that of the air; the most rapid rise takes place after the rectal temperature has reached 17°, when there may be a further rise to 32° in forty minutes; this is accompanied by an increase in the activity of the animal, and in the output of carbon dioxide.[5]

If the animal be fully awake and active, its temperature resembles that of a warm-blooded animal; a fall in external temperature increases its activity, temperature, and respiratory exchange, while a considerable rise has the opposite effect.[6]

The power of heat regulation in hibernating animals.—The capacity for maintaining a constant temperature varies according to the condition of the animal; during well-marked hibernation this power is very slight, and resembles that of a cold-blooded animal, but when the animal is active its power of regulating its temperature is comparable to that of a warm-blooded animal.

There is an intermediate stage when the animal is listless and inactive, with a bodily temperature below that of its normal in summer, but considerably above that of its surroundings. In this condition its power of regulation resembles that of an immature mammal; within certain narrow limits it is able to maintain its temperature, but when exposed to cold its temperature falls, and it passes into a cold-blooded condition.[7]

The awakening from hibernation.—One of the most interesting phenomena in hibernation is the sudden rise in temperature which occurs when the animal awakes from its torpor. This rise is so great and sudden that there is nothing comparable to it, not even the sudden rise seen in some cases of fever.

Thus Horvath[8] found the temperature of a sisel rise from 14° to 32° in one hour and forty minutes, the temperature of the air remaining 14°. In the

[1] In addition to other references, see Bernard, "Leçons sur la chaleur animale," 1876, p. 374.

[2] See references on pp. 794–795.

[3] *Compt. rend. Soc. de biol.*, Paris, 1894, 22 décembre.

[4] For further details see the works mentioned on p. 794; also Gavarret, "De la chaleur produite par les êtres vivants," Paris, 1855, p. 466.

[5] Horvath, *loc. cit.*; Pembrey and Hale White, *loc. cit.*

[6] Pembrey and Hale White, *loc. cit.*; Hunter, "Works," Palmer's Edition, London, 1837, vol. iv. pp. 141–145.

[7] Pembrey and Hale White, *loc. cit.*

[8] *Verhandl. d. phys.-med. Gesellsch. in Würzburg*, 1878, Bd. xii. S. 162.

bat and dormouse the rise may be even more rapid, as shown by the following examples:[1]—

Bat—Rectal temperature { = 17° when very quiet; = 34° when awake and active, fifteen minutes later } Temperature of air = 10°·5.

Dormouse— „ { = 13°·5 when asleep; = 35°·75 when awake and active, one hour later } Temperature of air = 9°·5.

The rapid rise in temperature is accompanied by a marked quickening of the respiration and of the heart-beat, and by active movements of the body. In some cases, especially in the marmot, there is a convulsive shaking of the body. The increase in the muscular activity appears to be the chief cause of the increased production of heat, although Horvath[2] and Dubois[3] do not accept this view. It is to be noted, however, that Horvath draws attention to the increased respiration and heart-beat, and remarks that when once the shivering movements of the marmot have commenced, nothing can prevent the animal from awaking, and its temperature from rising. Dubois considers that the liver plays the most important part, for he finds that extirpation of the ganglia of the solar plexus, or ligature of the portal vein, and of the inferior vena cava just above the liver, prevents the rapid rise of temperature observed in an awakening marmot. An examination, however, of the experiments made by Dubois shows that the influence of the nervous system is considerable, for the greater the motor paralysis the smaller was the rise in temperature.[4] Removal of the cerebral hemispheres does not prevent hibernation or the rise of temperature observed when the animal awakes. The latter phenomenon, however, is abolished by section of the spinal cord at the level of the fourth cervical vertebra.

In the case of bats and dormice, Pembrey and Hale White have shown that the sudden rise in temperature, when the animal awakes, is accompanied by a greatly increased discharge of carbon dioxide.

The causes of hibernation.—The cause generally assigned for hibernation is cold, but a more careful consideration of the facts long ago showed that cold could not be the sole cause of the phenomena. Most observers who have worked at the subject of hibernation have found that even severe cold will not cause an active animal to hibernate. Saissy[5] observed that a low temperature alone was ineffectual, but the continued effect of cold, and a limited amount of air for respiration, caused a marmot to pass into a typical hibernating condition even in summer. Mangili[6] found that torpid marmots and bats were awakened by exposure to severe cold, and that confined air would not cause hibernation. Valentin and Horvath[7] have recorded cases of marmots hibernating under normal conditions during summer; the animals were very fat, and the torpid condition was in all respects similar to that in winter. Pallas states that if the hamster be buried four or five feet below ground in a confined space, it begins to hibernate.[8]

Dormice have been kept throughout the winter in a warm room (16°), and yet they hibernated, and were not aroused when the external temperature

[1] Pembrey and Hale White, *loc. cit.*
[2] *Loc. cit.*, pp. 170, 175.
[3] *Compt. rend. Soc. de biol.*, Paris, 1893, pp. 210, 235; 1894, pp. 36, 115.
[4] *Ibid.*, 1893, p. 156.
[5] "Recherches expérimentales anatomiques," etc., 1808.
[6] *Arch. f. d. Physiol.*, Halle, 1808, Bd. viii. S. 433, 437, 444.
[7] *Verhandl. d. phys.-med. Gesellsch. in Würzburg*, 1881, Bd. xv. S. 209.
[8] See also Paul Bert, "Leçons sur la physiol. comp. de la respiration," Paris, 1870, p. 508.

was 20°; the warmth, however, delayed the onset of torpidity by two months, and made it less profound.[1] Further, it is found that in some cases hibernation takes place in the dry hot season; thus there is in Madagascar an animal, closely allied to the hedgehog, and called the tanrec (*Centetes ecaudatus*), which buries itself and becomes lethargic in the dry season, when its insect food is inaccessible.[2] The reptiles and many of the invertebrate animals of tropical climates seek their hiding-places and fall into a state of torpor during the dry season, when the heat is most intense. Torpidity in dormice and hedgehogs may be delayed or prevented by a plentiful supply of food.[3]

Want of food and cold seem to be the most important factors, but there must be some other condition, at present unknown, to explain the cases of marmots hibernating during the summer. It is certain that many species of animals which become torpid in one country do not become so in another. This fact, according to Barton,[4] is very noticeable in the United States, for many species which hibernate in Pennsylvania and other more northern parts of the country, do not hibernate in the Carolinas and other southern parts of the continent. Attempts have been made, but without success, to find anatomical differences, especially as regards the blood vessels of the brain, which would account for hibernation.[5]

The most recent theory is that of Dubois,[6] who maintains that hibernation is caused by an autonarcosis with carbon dioxide. In support of this theory he adduces the following facts, namely, the accumulation of carbon dioxide in the blood, and the production of torpidity in marmots exposed to an atmosphere containing about 40 per cent. of carbon dioxide.

The Influence of Various Conditions upon the Temperature of Man and Other Warm-blooded Animals.

Numerous observers have insisted upon the occurrence of small variations in the temperature of healthy men and animals, and have shown by experiments that these variations are due to several causes.

Influence of day and night.—The temperature of man is subject to slight daily variations; it rises during the morning and afternoon, it falls during the evening and early part of the morning. Upon the points of maximal and minimal temperature, and the range of variation, the results differ considerably, as the table on p. 799 shows.

It will be seen from these results that there is more agreement upon the time of the minimal daily temperature than upon that of the maximum. The causes of this difference are mainly two: in the first place, there appears to be a rise and then a fall in temperature before the ascent to the maximum begins. Thus Bärensprung found a rise in the early morning to 11 A.M., then a fall to 2 P.M.; and Damrosch observed that the temperature rose from 7 A.M. to 10 A.M., and then fell

[1] Berthold, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1837, S. 63.

[2] This statement of Cuvier and Bruguière is contested by Brown-Séquard ("Experimental Researches applied to Physiology and Pathology," New York, 1851, p. 25), who maintains that the tanrec hibernates in the winter season, when the external temperature is from 15 to 23 degrees.

[3] Reeve, "An Essay on the Torpidity of Animals," 1809.

[4] *Trans. Am. Phil. Soc.*, Phila., 1799, vol. iv. p. 121.

[5] Mangili, *Arch. f. d. Physiol.*, Halle, 1808, Bd. viii. S. 446; Saissy, *Deutsches Arch. f. d. Physiol.*, Halle, 1817, Bd. iii. S. 136.

[6] *Compt. rend. Acad. d. sc.*, Paris, 1895, tome cxx. p. 458; *Compt. rend. Soc. de biol.*, Paris, 1895, 3e Mars.

until 1 P.M. This small morning variation preceding the rise to the maximum would explain some of the uncertainty concerning the time of the maximum. The second important cause is the difference in the meals of the English and German people; the "*frühstuck*" is a small meal compared with the English breakfast, and thus, in the observations made in England, the morning fall, beginning about ten o'clock, would be masked by the increased warmth due to a hearty meal.

Time of Maximum.	Time of Minimum.	Range of Variations.	Place of Observation.	Observer.
Between 8 A.M. and 5 P.M.	About 1 A.M.	1°	Mouth.	Davy.[1]
About mid-day	Between 11 P.M. and 2 A.M.	0°·7	,,	Gierse.
At 7 P.M.	Between 11 P.M. and 8 A.M.	0°·72	,,	Hooper.
Between 10 A.M. and 7 P.M.	Between 11 P.M. and 7 A.M.	0°·73	,,	Hallmann.
Between 4 P.M. and 5 P.M.	Between 1 A.M. and 7 A.M.	0°·51 0°·56	,,	Lichtenfels and Fröhlich.
Between 4 P.M. and 7 P.M.	About 2 A.M.	0°·9	,,	Casey.
Between 4 P.M. and 8 P.M.	Between 12 P.M. and 7 A.M.	0°·5	,,	Clifford Allbutt.
About 7 P.M.	About 6 A.M.	0°·8	,,	Ogle.
Between 2 P.M. and 8 P.M.	Between 2 A.M. and 7 A.M.	0°·6	,,	Crombie.[2]
Between 9 A.M. and 6 P.M.	About 1 A.M.	1°·2	Axilla.	Ringer and Stuart.
Between 10 A.M. and 6 P.M.	Between 2 A.M. and 3 A.M.	1°·29	,,	Liebermeister.
About 5 P.M.	Between 7 P.M. and 7 A.M.	0°·4	,,	Damrosch.
At 3 P.M.	At 3 A.M.	1°·3	,,	Billet.
Between 6 P.M. and 7 P.M.	About 4 A.M.	0°·8	,,	Bärensprung.[3]
Between 4 P.M. and 9 P.M.	Between 2 A.M. and 8 A.M.	1°·3	Rectum.	Jurgensen.
Between 7 A.M. and and 7 P.M., generally about 4 P.M.	Between midnight and 4 A.M.	1°·4	,,	Jaeger.[4]
About 6 P.M.	About 2 A.M.	1°·25	,,	Nicol.
At 4 P.M.	At 7 A.M.	1°·2	Urine.	Richet.[5]
Between 5 P.M. and 8 P.M.	Between 3 A.M. and 6 A.M.	1°·4	,,	Pembrey.

Other causes for the different results are to be sought in the fact that the observations are not comparable as regards the age, health, meals, and work of the subjects of the experiment, and the temperature was taken in different ways.

The following curve (Fig. 76), given by Ringer and Stuart,[6] shows the daily fluctuations of temperature in a boy 12 years old; the thermometer, a non-registering one, was kept in the closed axilla throughout the

[1] The references are mostly given on p. 789 of this article.
[2] *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 550.
[3] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 159.
[4] Jaeger, *Deutsches Arch. f. klin. Med.*, Leipzig, 1881, Bd. xxix. S. 525.
[5] *Rev. scient.*, Paris, 1885, tome ix. pp. 430, 629.
[6] *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 187.

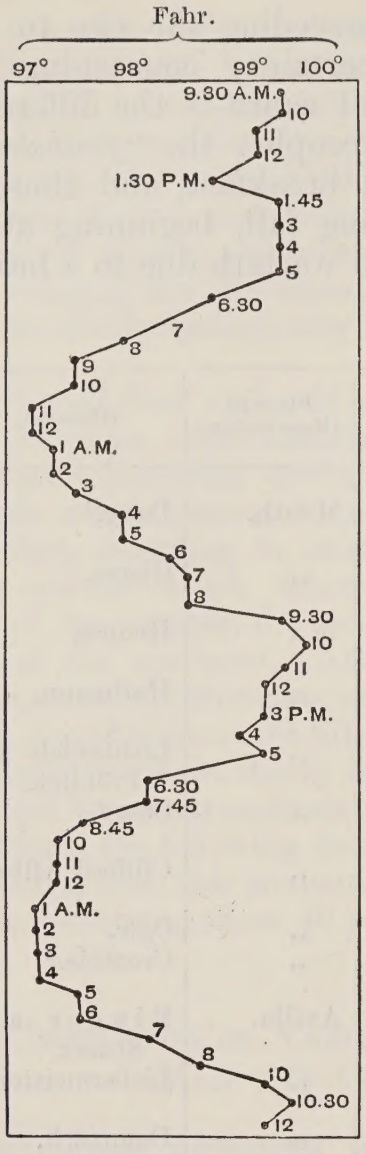


FIG. 76.—Daily variations in temperature observed by Ringer and Stuart. The observations extend over 50 hours.

time, and the readings were taken every hour. The boy was in good health, and was kept in bed during the observations.

In the next chart[1] (Fig. 77) are the daily curves representing the results of Ogle, Clifford Allbutt, Casey and Rattray, and those of Crombie, who, during residence in Bengal, made observations upon his own temperature and that of natives.

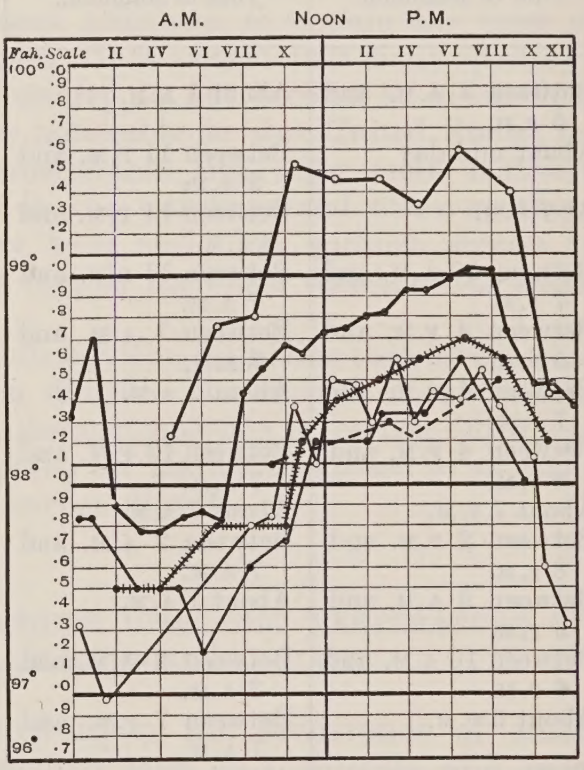


FIG. 77.—Daily variations in temperature observed by Ogle, Clifford Allbutt, Casey and Rattray, and Crombie.

The next curves (Fig. 78) represent the daily variation according to Jürgensen and Liebermeister's observations.[2]

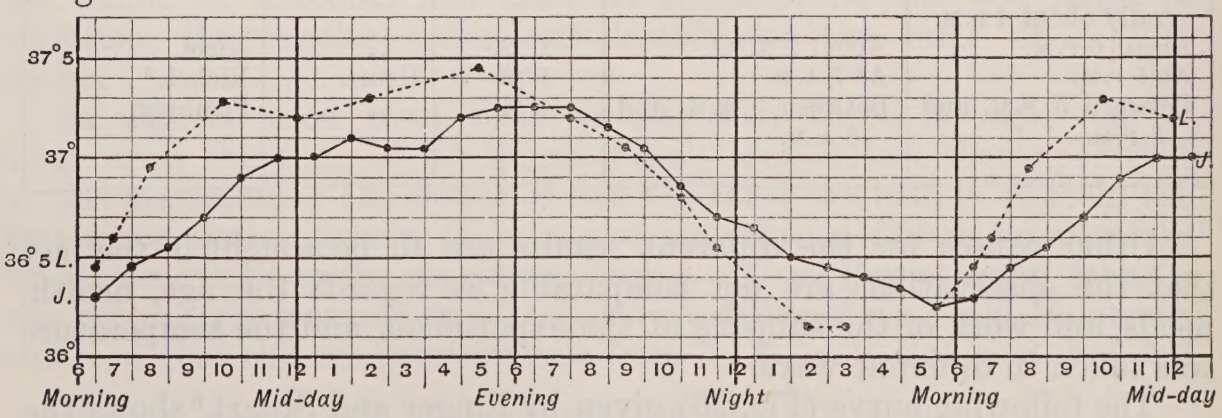


FIG. 78.—Daily variations in temperature observed by Jürgensen and Liebermeister. The observations extend over 30 hours.

The average results of Thierfelder's[3] observations upon the daily variations of temperature found in subjects of different age and sex are shown in the following table:—

[1] Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 568.
[2] "Handbuch der Pathologie und Therapie des Fiebers," Leipzig, 1875.
[3] *Schmidt's Jahrb.*, Leipzig, 1851, Bd. lxxi.

	Morning. (7–9 A.M.)	Noon.	Evening.
Newly born . . .	37°·41	37°·80	37°·61
Children . . .	37°·37	38°·07	37°·12
Adults { Men . .	37°·0	37°·25	36°·60
Adults { Women . .	37°·22	37°·55	37°·10
Aged	37°·25	37°·58	37°·31

The following curve[1] (Fig. 79) represents the mean results of records of the temperature of the urine taken by Richet, Gley, and Rondeau; the times of meals were 7 A.M., 11 A.M., and 7 P.M., and no observations were made between 9 o'clock in the evening and 7 o'clock in the morning.

Daily variations in temperature, similar to those already described, have been observed in natives of different races living in the tropics.[2]

FIG. 79.—Curve of daily variation in the temperature of the urine.

As regards the causes of the daily variation in temperature, muscular activity and food appear to be the most important factors. In ordinary life man is most active and takes food during the day, and is least active during the night. Debczynski[3] found that continuous work carried on throughout the night reversed the variation, so that the maximal temperature 37°·8 occurred in the morning, and the minimal 35°·3 in the evening. Night-watching without work had a similar but smaller effect, the maximal temperature 37°·7 being in the morning, the minimal 37°·5 in the evening. Jaeger[4] has obtained similar results, and Krieger[5] states that work during the night and rest during the day reverse the daily variation. The influence of inversion of the ordinary routine of daily life has been studied by U. Mosso[6]; a series of observations of the rectal temperature was first made during a period when work was performed in the daytime and sleep taken at night, and the two chief meals were at 11 A.M. and 6 P.M.; then there followed another period in which sleep was taken during the day and work performed at night, and

[1] Richet, *Rev. scient.*, Paris, 1885, tome ix. p. 430.

[2] Davy, "Researches," London, 1839, vol. i. p. 169; Jousset, *Arch. de méd. nav.*, Paris, 1883, tome xl. p. 124; Maurel, *Bull. Soc. d'anthrop. de Paris*, 1884, tome vii. p. 381.

[3] *Jahresb. ü. d. Leistung. . . . d. ges. Med.*, Berlin, 1875, Bd. i. S. 248.

[4] Jaeger, *Deutsches Arch. f. klin. Med.*, Leipzig, 1881, Bd. xxix. S. 533.

[5] *Ztschr. f. Biol.*, München, 1869, Bd. v. S. 479.

[6] *Arch. ital. de biol.*, Turin, 1887, tome viii. p. 177.

the two chief meals were at 11 P.M. and 6 A.M. Notwithstanding the inversion of daily routine, Mosso found that the morning rise still took place about the same time, and, as the following curves (Fig. 80) will show, the daily variation was not inverted, although the sleep during the day caused a fall, and getting up in the evening a marked rise, in temperature. The effect of the experiment was to disturb the regularity of the daily variation, but on the fourth day the influence of the sleep during the day was most marked, a fact which seems to indicate that, if the habit were long continued, a tendency to inversion would be observed in the daily variation of temperature.

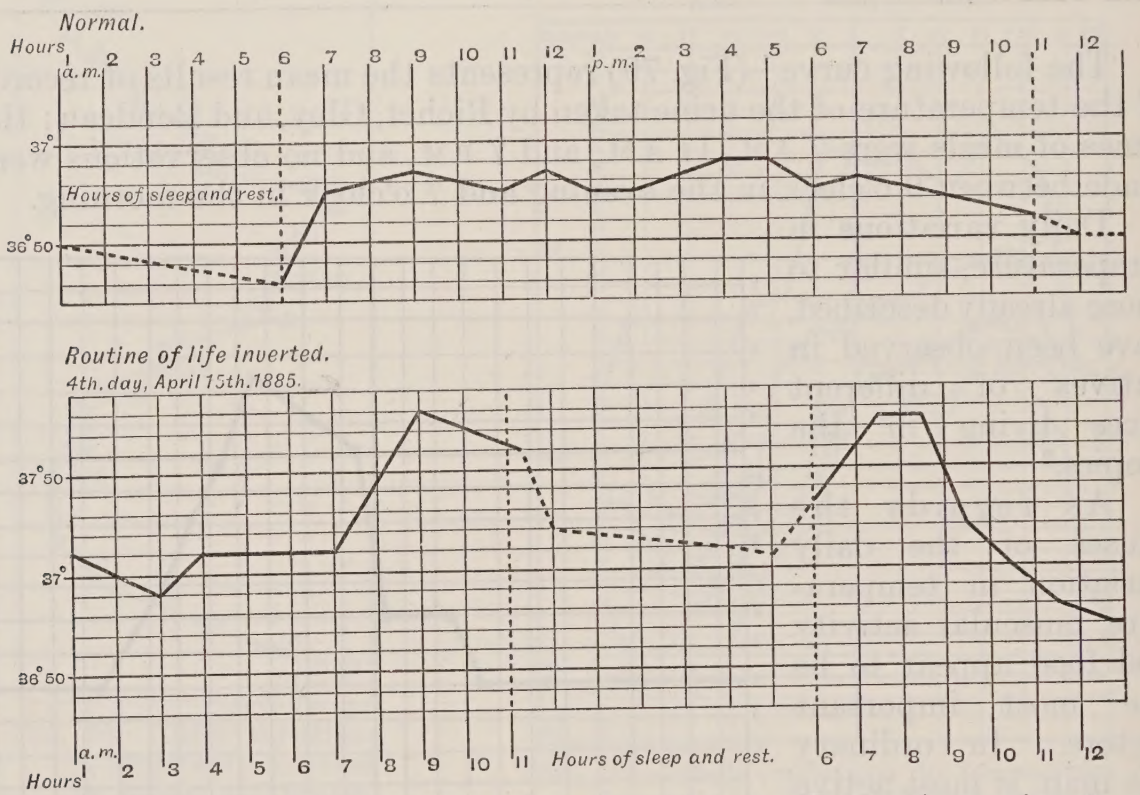


FIG. 80.—Daily variations in temperature observed during U. Mosso's experiments.

Buchser,[1] an engineer, who was accustomed to sleep during the day and work at night, found that his average morning temperature was 37°·25, while his evening temperature was 36°·8.

There are secondary causes of the daily variation. The periods of high and low bodily temperature more or less correspond with the times of day when the external temperature is high and low respectively. Further, there appears to be a certain periodicity, the result of long-continued habits of life, stamped upon the processes which regulate temperature. This is shown by the fact that the daily variation still persists, although it may be slightly modified, during a period of fasting or night-watching,[2] and a similar daily variation is observed in the respiration and pulse,[3] in the discharge of urea,[4] and in the capacity

[1] Quoted from Carter, *Journ. Nerv. and Ment. Dis.*, N.Y., 1890, vol. xvii. p. 785.

[2] Jürgensen, "Die Körperwärme des gesunden Menschen," Leipzig, 1873; Ogle, *St. George's Hosp. Rep.*, London, 1866, vol. i. p. 228; Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 597; Liebermeister, "Handbuch d. Path. u. Therap. des Fiebers."

[3] Lichtenfels and Fröhlich, *Denkschriften d. k. Akad. d. Wissensch. Wien*, 1852, Bd. iii. Abth. 2, S. 113; Neuhauss, *Virchow's Archiv*, 1893, Bd. cxxxiv. S. 365. See also this article, p. 813; Bosanquet, *Lancet*, London, 1895, vol. i. p. 672; Damrosch, *Deutsches Arch. f. klin. Med.*, Leipzig, 1853, S. 342; Jousset, *Arch. de méd. nav.*, Paris, 1883, tome xl. pp. 284-5; Chossat, *Mém. Acad. d. sc. de l'Inst. de France*, Paris, 1843, tome viii. p. 540.

[4] Weigelin, *Arch. f. Anat., Physiol. u. wissensch. Med.*, Leipzig, 1868, S. 207.

for muscular work.[1] Daily variations in the output of carbon dioxide and in the intake of oxygen have been observed by Prout, Pettenkofer and Voit, Fredericq,[2] and others; these variations in metabolism more or less correspond with those observed in the temperature, and will be found discussed more fully in another part of this work.[3]

Rest in bed throughout the day does not abolish the daily variation; it is still present, although modified, in cases of disease, attended or unattended by fever; the morning rise still takes place even when light is excluded (Ogle).

In animals, daily variations in temperature have also been observed, but upon this point there are few exact observations taken throughout the day and night. Strecker,[4] from observations upon 150 horses, found the average temperature between 6.30 A.M. and 8 A.M. to be 37°·9, that between 5 P.M. and 6.30 P.M. to be 37°·93; but the minimum was 37°·2 and the maximum 38°·6. In the case of oxen, Robertson [5] found the average morning temperature 38°·7, the evening temperature 38°·9; in the cat the minimum is 37°·8 at 7 A.M. and the maximum 39°·08 at 10 P.M. (Bidder and Schmidt).[6] Hunter states that the temperature of an ass was 0°·5 higher in the evening than in the morning. According to the observations of Siedamgrotzky,[7] the maximal daily temperature in horses was 38°·2 at 6 P.M., the minimum 37°·5 at 4 A.M.; in a cow the maximum was 39°·1 at 5 P.M., and the minimum 38°·7 at midnight. Corin and Van Beneden [8] have observed the daily variation in pigeons,[9] and find that the minimum is at 4 A.M.; that from this time to 8 A.M. there is a rise, then a fall to noon, followed by a rise to the maximum at 4 P.M.; the daily variation amounts to 2°·2. In the case of horses, Hobday [10] finds that the rectal temperature at 10 A.M. is 37°·6, and 37°·9 at 5 P.M.; in the case of the rabbit, cat, and dog, Carter [11] has shown that there is a distinct rhythm of temperature, the maximum occurring in the evening (7–11 P.M.) and the minimum in the morning (7–11 A.M).

We may conclude that the daily variation in temperature is one of the features of a corresponding variation in the activity of the tissues of the body, as shown by the rate of the contraction of the heart, the frequency of respiration, the intake of oxygen, the output of carbon dioxide, the discharge of urea, and the capacity for muscular work.

The influence of age.—The temperature of newly-born infants and animals is generally equal to, or even slightly higher than, that of their parents, but it is much less stable, and is liable to much greater variations.

Edwards [12] found that the temperature of newly-born pups, kittens, and rabbits fell when they were removed from their warm surroundings, and continued to fall until it reached a point a few degrees above the

[1] Patrizi, *Arch. ital. de biol.*, Turin, 1892, tome xvii. p. 134.
[2] Prout, *Ann. Phil.*, London, 1813, vol. ii. p. 330; vol. iv. p. 331; Pettenkofer and Voit, *Ztschr. f. Biol.*, München, 1866, Bd. ii. S. 459; Fredericq, *Arch. de biol.*, Gand, 1882, tome iii. p. 729.
[3] "Chemistry of Respiration," this Text-book, vol. i. p. 721.
[4] Ellenberger, "Vergleichende Physiologie der Haussäugethiere" 1892, Bd. ii. Th. 2, S. 81.
[5] *Veterinary Journ.*, London, 1885, vol. xx. p. 311.
[6] "Die Verdauungssäfte und der Stoffwechsel," Leipzig, 1852, S. 346.
[7] *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1875, Bd. i. S. 87.
[8] *Arch. de biol.*, Gand, 1887, tome vii. p. 265.
[9] See also Chossat, *Mem. Acad. d. sc. de l'Inst. de France*, Paris, 1843, tome viii. p. 540.
[10] *Journ. Comp. Path. and Therap.*, Edinburgh and London, 1896, vol. ix. p. 286.
[11] *Journ. Nerv. and Ment. Dis.*, N.Y., 1890, vol. xvii. p. 782.
[12] "De l'influence des agens physiques sur la vie," Paris, 1824.

temperature of the air. Newly-born guinea-pigs, however, were able to maintain their temperature, provided that the exposure to cold was not very great. Edwards therefore divided the young warm-blooded animals into two classes, the warm-blooded and the cold-blooded. In the former class the young animals are at birth blind, helpless, in some cases naked, and cannot maintain their temperature. The members of the latter class are even at birth in a condition of great development; their eyes are open, they are active, and maintain a fairly constant temperature. It was also found that young birds could be classified in a similar manner. As the animal grows, the fall in temperature on exposure becomes less and less, and about the fifteenth day after birth a fairly constant temperature can be maintained.

Edwards showed by comparative experiments that the fall in temperature on the exposure of newly-born animals was not due to the greater cutaneous surface, in proportion to the mass of the body, as compared with the ratio in adults. The absence or presence of feathers or fur was only of secondary import, for an adult sparrow was able to maintain its temperature even after all its feathers had been plucked out.

Raudnitz[1] in 1888 discussed very fully the temperature of infants. He made observations upon the variations of temperature in infants at birth and during the first few days after birth. The influence of the large cutaneous surface in relation to the mass of the body, and the loss of heat from the skin, were shown by experiment to be only secondary causes of the irregular temperature. Observations made upon the effect of affusions of cold water showed that the rectal temperature in infants a day or two old rose in the case of strong subjects, but remained stationary or fell in the case of the weak. Raudnitz concludes that the imperfect development of the power of regulating temperature is the chief cause of the variable temperature in infants; and it has been shown by the writer[2] that this is also the cause in the case of young immature animals.

Before birth the temperature of the infant is slightly higher than that of the mother's uterus;[3] at birth the average rectal temperature is 37°·5 (99°·5). Soon after birth, especially after the first bath, the temperature falls to about 36°·75 (98°·15), and during the next week or two rises somewhat, and remains fairly constant between 37°·25 (99°·05) and 37°·6 (99°·68). These figures are to be looked upon only as average results, for all observers appear to agree that the daily fluctuations of temperature are greater and more uncertain in children than in adults.[4]

[1] *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 423. At the end of this paper is a very complete list of papers bearing upon the subject.

[2] Pembrey, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 363.

[3] Wurster, *Berl. klin. Wchnschr.*, 1869, Nr. 37; Alexceff, *Arch. f. Gynaek.*, Berlin, Bd. x. S. 141; Fehling, *ibid.*, Bd. vii. S. 146; Preyer, "Specielle Physiologie des Embryo," Leipzig, 1885, S. 362.

[4] Bärensprung, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 138; Finlayson, "On the Normal Temperature of Children," *Glasgow Med. Journ.*, 1869, p. 186; Squire, *Trans. Obst. Soc. London*, vol. x. p. 274; Raudnitz, *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 423; here other references will be found; Jürgensen, "Die Körperwärme des gesunden Menschen," 1873, S. 49; Davy, "Researches," London, 1839, vol. i. p. 156; Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 594; Mignot, Thèse de Paris, 1851; Wurster, *Berl. klin. Wchnschr.*, 1869, Bd. vi. S. 37; Andral, *Compt. rend. Acad. d. sc.*, Paris, 1870, p. 815; Roger, *Arch. gén. de méd.*, Paris, Sér. 4, tome v. p. 273; "De la temperature chez les enfants," Paris, 1844; Lépine, *Gaz. méd. de Paris*, 1870; Fehling, *Arch. f. Gynaek.*, Berlin, 1874, Bd. vi. S. 385.

The average temperature falls one- or two-tenths from infancy to puberty, and about the same amount from puberty to middle age; after that stage is reached the temperature rises, and about the eightieth year is almost as high as in infancy.[1] According to Ringer and Stuart,[2] the average daily maximum in persons under 25 years is 37°·2 (99°), in those over 40 years, 37°·1 (98°·8).

As regards the temperature in old age, all observers seem to agree that it is equal to or slightly above that of adults. Davy[3] found the mean temperature of eight healthy old persons, with an average age of 88, to be 36°·9 (98°·45) in the mouth. Charcot[4] states, as the result of numerous determinations, that the rectal temperature in the aged is 37°·2 to 37°·5, and is rarely higher or lower than in the adult; but the temperature in the well-closed axilla is often two or three degrees below that in the rectum, on account of the small and feeble circulation in the skin of the aged. Mossé and Ducamp[5] have compared the temperature of the axilla and rectum of aged people, and have obtained the following results; each figure represents the mean of twelve or fifteen observations:—

Age.	Morning Temperature.		Evening Temperature.	
	Axilla.	Rectum.	Axilla.	Rectum.
75	36°·40	36°·83	36°·58	37°·04
76	36°·48	37°·06	36°·41	36°·86
80	36°·08	36°·46	36°·40	36°·94

The results obtained by Roger[6] upon seven healthy people, whose ages ranged between 72 and 95 years, are, for the mean temperature, 36°·68 and 36°·23; for the minimum 36° and 35°·5, for the maximum 37°·10 and 37°, in the axilla and mouth respectively.

In the case of young animals born in an advanced condition of development, the temperature is generally higher than that of the parents. Thus foals and calves, several hours after birth, have a temperature 0°·5 to 1° above that of their mothers. The average temperature of foals for the first five days is 39°·3, and then gradually falls, as shown by the table on p. 806, which represents the results of six hundred observations made by Föhringer[7] upon one hundred horses.

Similar results as regards the effect of age in horses were obtained by Siedamgrotzky,[8] and in the case of cows and sheep by Hobday.[9]

[1] Wunderlich, "Medical Thermometry"; Bärensprung, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 148.

[2] *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 194.

[3] *Phil. Trans.*, London, 1844, pt. 1, p. 59; "Researches," London, 1839, vol. iii. p. 4.

[4] *Gaz. hebd. de méd.*, Paris, 1869, tome vi. p. 324.

[5] *Gaz. hebd. d. sc. méd. de Montpellier*, 1886.

[6] *Arch. gén. de méd.*, Paris, Sér. 4, tome v. p. 273.

[7] Ellenberger, "Vergleichende Physiologie der Haussäugethiere," 1892, Bd. ii. Th. 2, S. 81.

[8] *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1875, Bd. i. S. 87.

[9] *Journ. Comp. Path. and Therap.*, Edinburgh and London, 1896, Bd. ix. p. 286.

Age.	In the Stables.	Age.	In the Fields.
4–6 years	38°·05	4–6 years	37°·40
6–8 ,,	37°·92	6–12 ,,	37°·24–37°·49
8–18 ,,	37°·85	12–18 ,,	37°·48

The influence of muscular work.—During muscular work there is an increased production of heat, and were it not for the compensation brought about by the increased loss of heat the temperature of the body would rise considerably. The effect, therefore, of muscular work upon the mean temperature varies according to the perfection of the compensation. Jürgensen[1] found that the work involved in sawing wood for six hours raised the temperature of a healthy man 1°·2 above the normal, but as soon as the work was finished the temperature fell rapidly. Davy[2] made numerous observations upon the effect of active exercise on his own temperature. The highest readings of the thermometer under the tongue were 37°·5 (99°·5) and 37°·8 (100°); some previous observations upon the temperature of men after walking two or three hours showed a rise of ·8° in the temperature of the urine, but no change in that taken in the mouth; after a rest the temperature rapidly fell to the normal. Alpine climbing, even on cold days, was found by Clifford Allbutt[3] to raise the temperature of the mouth about half a degree; the same form of exercise was taken by Liebermeister and Hoffmann,[4] who observed the temperature in the axilla during both the ascent and descent; the chief results were as follows:—

Liebermeister's temperature,		36°·82	before ascent and			37°·85	maximum during ascent.		
Hoffmann's	,,	36°·50	,,	,,	,,	37°·95	,,	,,	,,
Liebermeister's	,,	36°·60	,,	descent	,,	37°·60	,,	,,	descent.
Hoffmann's	,,	36°·40	,,	,,	,,	37°·25	,,	,,	,,

Results directly opposed to the above have been obtained by Lortet,[5] whose observations were made on level ground and during two ascents of Mont Blanc (4810 metres high) in August 1869. On level ground Lortet found that, when he was at rest, the temperature of his mouth was 36°·4, but 36°·2 during bodily exercise. During the ascents of Mont Blanc the temperature fell progressively and even reached as low a point as 31°·8, but after a few minutes' rest it rapidly reached the normal. Lortet explained these results by saying that during work the chemical forces which would have sufficed in the rarefied atmosphere to maintain the normal temperature of the body, were partly resolved in motion, and therefore the temperature fell. These results have been criticised by Clifford Allbutt and Liebermeister, and there can be little doubt but that the low temperatures observed were due to the cooling of the thermometer in the mouth by the laboured breathing of the cold air, which was sometimes several degrees below zero. This criticism[6]

[1] "Die Körperwärme des gesunden Menschen," Leipzig, 1873, S. 43–46.
[2] *Phil. Trans.*, London, 1844, pt. 1, p. 62; 1845, pt. 2, p. 322; 1850, p. 440.
[3] *Journ. Anat. and Physiol.*, London, 1872, vol. vii. p. 106.
[4] Liebermeister, "Handbuch der Path. u. Therap. des Fiebers," 1875, S. 84.
[5] *Compt. rend. Acad. d. sc.*, Paris, 1869 p. 709.
[6] These sources of error have been shown to exist, for Arkle (experiments made at the request of the writer, and the results of which will be published later), during mountain climbing in the summer of 1897, found a constant rise of two or three degrees in the rectal temperature, but the mouth gave a low temperature. In fact, it was impossible to obtain accurate results by placing the thermometer in the mouth.

is further supported by the fact that Lortet found a few minutes' rest sufficient to raise the temperature to the normal.

Marcet,[1] shortly before Lortet's observations, found that during an ascent of some of the Mont Blanc chain of mountains the temperature of his mouth fell. This result was contested by Vernet, who had determined the rectal temperature under similar circumstances, and, as the result of the controversy, Marcet and Vernet[2] in 1888 ascended together one of the highest points of the Jura. They found that there was a distinct rise in the rectal temperature. Marcet, however, does not look upon this result as conclusive; he attempts to explain the rise of temperature as due to congestion of the hæmorrhoidal vessels. It must be pointed out, however, that the increased circulation due to exercise would probably not cause congestion, and, whether it did or not, the rise in the temperature of the rectum indicates a rise in the temperature of the internal parts of the body. Further, Marcet himself shows that cooling the under surface of the chin causes a fall in the temperature of the mouth, and this was probably the cause of the low readings observed in his first ascents.

Obernier[3] found that a walk for thirty-five minutes, when the external temperature was 11°·2, raised the rectal temperature from 37° to 38°. A walk of five miles raised the temperature of Ogle's mouth from 37° to 37°·45.[4] Similar results have been obtained by others.[5]

Similar results to the above have been obtained upon animals. The temperature of a dog during the first hour of work upon a treadmill was raised 1°·8, but although the work was continued the temperature quickly fell (U. Mosso).[6] In the case of two stallions three years old, Liska[6] found the temperature before work 37°·8 and 38°·0 respectively; after fifteen minutes' work, 39°·5 and 39°; and again, after twenty minutes' rest, 37°·7 and 38°. Siedamgrotzky[7] found that exercise raised the temperature of horses by an amount varying from 0°·3 to 1°, while Hobday[8] found in the case of healthy omnibus horses that the rectal temperature was generally raised 2° or more by hard work, and in sheep and pigs the exertion of running caused a similar rise in temperature.

Further details of the production of heat in muscle will be given later.

In the case of insects the effect of muscular activity is very marked. Thus Newport[9] found the temperature of the abdomen of a very active humble-bee (*Bombus terrestris*) to be 23°, when the air was 19°·3; four of these active bees placed in a glass bottle raised the temperature of the air from 19°·3 to 23°·6.

The influence of mental work.—Mental activity is said to have an effect both upon the general temperature of the body and upon the local temperature of the brain and head. Thus Davy[10] found that mental

[1] *Arch. d. sc. phys. et nat.*, Genève, tome xxxvi. p. 247.

[2] Marcet, Croonian Lectures, *Brit. Med. Journ.*, London, 1895, vol. i. p. 1367.

[3] "Der Hitzschlag," Bonn, 1867, S. 80.

[4] *St. George's Hosp. Rep.*, London, 1866, vol. i. p. 232.

[5] Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 579; Roger, "Recherches cliniques sur les maladies de l'enfance," tome i. p. 227; Speck, *Arch. d. Ver. f. gemeinsch. Arb. z. Förd. d. wissensch. Heilk.*, Gottingen 1862, Bd. vi. S. 161–324; Cuny Bouvier, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 386.

[6] Ellenberger, "Vergleichende Physiologie der Haussäugethiere," 1892, Bd. ii. Th. 2, S. 87.

[7] *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1875, Bd. i. S. 87.

[8] *Journ. Comp. Path. and Therap.*, Edin. and London, 1896, vol. ix. p. 286.

[9] *Phil. Trans.*, London 1837, pt. 2, p. 259.

[10] *Ibid.*, 1845, pt. 2, p. 319; 1850, p. 443.

work in England and in the tropics raised his temperature 0°·27 and 1°·1 respectively, and an increase varying from 0°·1 to 0°·7 has been observed after similar exertion by Speck,[1] Rumpf,[2] and Gley[3]; the temperature was taken in the rectum, axilla, or mouth. Clifford Allbutt,[4] however, in a long series of observations, found that mental work had no effect upon the temperature.

Cavazzani[5] states that in the case of a man whose skull had been trephined over the right temporo-occipital region, a thermometer placed in the dura mater showed a rise of two-tenths of a degree during mental activity. A. Mosso[6] maintains that intense psychical processes may cause so much heat to be set free in the brain that its temperature may remain for some time 0°·2 to 0°·3 above the temperature of the rectum. In a curarised dog the action of cocaine may produce a rise of as much as 4° in the temperature of the brain (37° to 41°). In man, Lombard[7] found that mental activity caused a slight rise in the temperature of the head, especially in the occipital region.

It is probable, however, that this local rise of temperature is not due, as Mosso believes, to very active combustion in the ganglion cells, but to vascular changes consequent upon the mental activity. Hill and Nabarro,[8] have shown that the blood from the venous sinuses of the skull is less venous in colour than that of the femoral vein, that the metabolism of the brain is very low, and that it is scarcely increased during an epileptic fit. The average differences between the gases in samples of blood from the carotid artery and from the torcular Herophili of dogs were as follows:—

	Normal.			Tonic Fit.			Clonic Fit.		
	Art.	Torc.	Diff.	Art.	Torc.	Diff.	Art.	Torc.	Diff.
Carbon dioxide	40·86	44·74	+3·87	44·98	49·04	+4·06	30·59	33·58	+2·99
Oxygen . .	16·81	13·39	−3·42	15·17	10·22	−4·95	15·77	11·46	−4·31

It is probable, therefore, that the temperature of the brain is not perceptibly greater than that of the blood. The cerebral circulation changes passively with every alteration of the general arterial or venous blood pressure,[9] and this is apparently the explanation of Lombard and Mosso's results. Moreover, the experiments of Helmholtz,[10] Heidenhain,[11] and Rolleston[12] have failed to demonstrate the formation of heat in nerve.

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1882, Bd. xv.
[2] *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiii. S. 601.
[3] *Compt. rend. Soc. de biol.*, Paris, 1884, p. 265.
[4] Note communicated to the writer.
[5] *Arch. ital. de biol.*, Turin, 1893, tome xviii. p. 328.
[6] *Proc. Roy. Soc. London*, 1892, vol. li. p. 83; "Die Temperature des Gehirns," Leipzig, 1894.
[7] *Arch. de physiol. norm. et path.*, Paris, 1868, tome i. p. 670.
[8] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 218.
[9] Roy and Sherrington, *ibid.*, 1890, vol. xi. p. 85; Hill, *ibid.*, 1895, vol. xviii. p. 15.
[10] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1848, S. 158.
[11] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, Bd. iv. S. 250.
[12] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 208.

The influence of food.—The investigations of many observers[1] show that the effect of food upon the temperature of the body is to cause a slight rise, or, in the case of the evening meals, to postpone for a short time the customary fall of temperature at that time. The rise is often inappreciable and rarely exceeds half a degree; the maximal effect is seen about one hour and a half after the meal. A draught of cold water (10°) lowers the temperature about half a degree.[2]

In the case of the horse the effect of food is to cause a rise of 0°·2 to 0°·8, which persists for three or four hours.

Maurel[3] states that in the rabbit food is the chief cause of the daily variation in temperature, for if the animal be kept without food during the day but be fed during the night, the temperature shows a rise to the maximum, not at the usual time, in the evening, but in the morning. This is denied by Carter,[4] who observed an evening rise in the temperature of rabbits which had fasted three days.

Bernard[5] determined the temperature of the blood of the portal and hepatic veins under different conditions as regards the nutrition of the animals, and came to the conclusion that more heat was produced in the liver during digestion. The following are some of his results:—

	Blood of Portal Vein.	Blood of Hepatic Vein.	Blood of Right Side of Heart.
Dog—After fasting for four days .	37°·8	38°·4	38°·8
,, Beginning of digestion . .	39°·9	39°·5	...
,, In full digestion . . .	39°·7	41°·3	39°·2

The effect of starvation upon the temperature of animals has been studied chiefly by Chossat,[6] and Bidder and Schmidt.[7] The first observer made experiments on twelve pigeons, and he found that the rectal temperature gradually fell until a short time before death; during the period of inanition the daily variation in temperature became more marked, and towards the end of life a rapid fall in temperature occurred. The results are shown in the table on p. 810.

On the day of death the temperature of the pigeon fell to 26°·2. Similar experiments on turtle-doves, hens, rooks, rabbits, and guinea-pigs gave the following temperatures:—22°·9, 28°·2, 34°·3, 27°·0, and 23°·9 respectively on the day of death.

Bidder and Schmidt experimented upon a cat, and found that after

[1] Davy, *Phil. Trans.*, London, 1845, pt. 2, p. 319; *ibid.*, 1850, p. 444; Damrosch, *Deutsche Klinik*, Berlin, 1853, S. 317; Jürgensen, "Körperwärme des gesunden Menschen," Leipzig, 1873, S. 21; *Deutsches Arch. f. klin. Med.*, Leipzig, 1867, Bd. iii. S. 165; Ringer and Stuart, *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 194; Ogle, *St. George's Hosp. Rep.*, London, 1866, vol. i.; Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 581.

[2] Liebermeister, "Handbuch d. Path. u. Therap. des Fiebers," Leipzig, 1875, S. 123; Wunderlich, "Medical Thermometry"; Siedamgrotzky, *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1875, Bd. i. S. 87.

[3] *Compt. rend. Soc. de biol.*, Paris, 1884, p. 588.

[4] *Journ. Nerv. and Ment. Dis.*, N.Y., 1890, vol. xvii. p. 785.

[5] "Leçons sur la chaleur animale," Paris, 1876.

[6] "Recherches expérimentales sur l'inanition," Paris, 1843, quoted from Gavarret, "De la chaleur etc.," p. 394.

[7] "Die Verdauungssäfte und der Stoffwechsel," Leipzig, 1852, S. 322.

355 hours' hunger the rectal temperature fell from 39°·08 to 38°·4; after 369 hours, to 38°·1; after 393, to 35°·5; after 415 hours, to 33°·7; and again, after 426 hours, to 32°·4, when the animal died.

	Rectal Temperature.		Daily Variation.
	Midday.	Midnight.	
Normal pigeons	42°·22	41°·48	0°·74
Complete inanition, first period .	42°·1	39°·8	2°·3
,, ,, second ,, .	41°·9	38°·7	3°·2
,, ,, third ,, .	41°·4	37°·3	4°·1

In the case of the fasting man Tanner,[1] no fall in temperature was observed after thirty days' fast; the temperature of his mouth was 36°·9 (98°·4) on the twenty-fifth day, and 37°·1 (98°·8) on the thirtieth day. It is uncertain whether the fast was perfectly genuine, for Tanner took a certain amount of liquid. Noyes[2] recorded a temperature of 34°·4 (94°) in the case of a partly demented man, who had taken no food for forty-five days, but it is to be noted that the condition was complicated by paralysis of the lower limbs.

The influence of sleep.—The heat of the body falls during the night and early morning, the time of inactivity and rest, but, according to Bärensprung[3] and Wunderlich,[4] sleep in itself has no influence on the temperature. Crombie,[5] on the other hand, found that sleep during the day caused a fall in temperature of about half a degree, but was rapidly followed by a rise after awaking. Hunter[6] found that during sleep the temperature fell about eight-tenths of a degree. The observations of Jürgensen and Liebermeister[7] show that the temperature of a man asleep is not lower than his temperature at a similar time of day when he is awake and lying still. Inactivity causes a fall in temperature, and sleep is a condition in which inactivity is most marked. Liebermeister[8] found that, by contracting the habit of sleeping each afternoon for ten days, the mean temperature of his axilla fell to about 36°·5, whereas it had previously been for that time of day 37°·3. Observations by U. Mosso[9] also show that sleep during the daytime causes a fall in the rectal temperature of man.

The influence of sex.—Very little difference in temperature can be observed in the two sexes.[10] Women may have a slightly higher temperature, but the difference does not exceed half a degree; their temperature, however, appears to be more liable to variations. Davy[11]

[1] *Brit. Med. Journ.*, London, 1880, vol. ii. p. 171. [2] *Ibid.*, p. 557.
[3] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 163.
[4] "Medical Thermometry," p. 109.
[5] *Loc. cit.*, p. 585.
[6] *Phil. Trans.*, London, 1778, vol. lxviii. pt. 1, p. 20; "Works," Palmer's edition, London, 1837, vol. iv. p. 144.
[7] Liebermeister, "Handbuch d. Path. u. Therap. des Fiebers," 1875, S. 87.
[8] *Ibid.*, S. 92.
[9] *Arch. ital. de biol.*, Turin, 1887, tome viii. p. 177. See also this article, p. 802.
[10] Wunderlich, "Medical Thermometry."
[11] *Med. Times and Gaz.*, London, 1864, vol. ii. p. 337.

concluded that the temperature of women and female animals was lower than that of the male, but his observations were made upon only three or four individuals. Thus he states that the temperature of three healthy men varied between 37°·2 and 37°·5, that of three women between 36°·5 and 36°·7; in the case of three cocks and three hens the results were 42°·4 and 42°·1 respectively. Bärensprung[1] found no marked difference, the average temperature of eighteen women being 37°·25. As the result of seventy or eighty observations, Siedamgrotzky[2] gives the temperature of stallions, mares, and geldings as 37°·8, 38°·2, and 38°·05 respectively; the average temperature of a large number of ducks was found by Martins[3] to be 41°·96 for the male, and 42°·27 for the female. Singleton[4] determined the rectal temperature of fifty dogs and of fifty bitches; the average for the former was 38°·9, for the latter 38°·7. The observations were made at similar times of the day, but upon animals of different breeds.

The influence of race.—The natives of tropical countries appear to have a temperature slightly higher than that observed in the inhabitants of mild or cold climates, but the difference is to be ascribed mainly to the climate. Davy,[5] from observations made upon natives in the Cape of Good Hope, Isle of France, and Ceylon, found the temperature to be about 0°·6 higher than the average in temperate climates; Crombie[6] made fifty-two observations on Hindus, Mohammedans, and East Indians in Bengal, and found that the average temperature from 10 A.M. to 10 P.M. was between 37°·2 and 37°·8, that from 10 P.M. to 10 A.M. between 36°·7 and 37°·2. Both of these observers also found that the temperature of Europeans living in the same district was about half a degree higher than the average in England. Jousset[7] made numerous observations on natives and Europeans living in tropical climates, and came to the conclusion that the axillary temperature is generally 0°·7 to 0°·8 higher than that observed in temperate climates. The following figures show that climate, and not race, is the important factor:—

Natives of Tropics.		Europeans.	
Hindus . . .	37°·85	Officials at Chandernagore .	38°·16
Cochin-Chinese . .	37°·60		
Chinese . . .	37°·85		
Negroes of Senegal .	37°·70	Sailors at Senegal . .	37°·75
,, Congo .	37°·80	,, Antilles . .	37°·70
,, Antilles .	37°·80	Soldiers at ,, . . .	37°·75

Similar results to the above were obtained by Maurel.[8]

The temperature of natives in South Africa was found by Livingstone[9] to be 36°·7, when the temperature of the air in the shade was 42°·2,

[1] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 155.
[2] *Deutsche Ztschr. f. Thiermed.*, Leipzig, 1875, Bd. i. S. 87.
[3] Ellenberger, "Vergleichende Physiol. der Haussäugethiere," 1892, Bd. ii. Th. 2, S. 85.
[4] See p. 790.
[5] "Researches," London, 1839, vol. i. p. 169.
[6] *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 591.
[7] *Arch. de méd. nav.*, Paris, 1883, tome xl. pp. 123, 426.
[8] *Bull. Soc. d'anthrop. de Paris*, 1884, tome vii. p. 380.
[9] "Travels and Researches in South Africa," 1857, p. 509.

but his own temperature was 37°·8, owing probably to the difference in clothes. Thomson[1] found the mean temperature of natives in Iceland to be 37°·27, and Eijkman[2] states that the average temperature of Europeans living in Batavia is 37°·02, that of the Malays 36°·93.

The influence of menstruation and pregnancy.[3]—Normal menstruation and pregnancy in healthy women have no marked influence upon the general temperature of the body. During labour the temperature rises somewhat during the pains, but falls again between the pains. Immediately after delivery a slight fall in temperature occurs.

Individual peculiarities in temperature.—Observations on men, and especially on animals, show that the mean temperature of different individuals is not the same, even when the conditions are as far as possible equal.[4] The mean temperature in the axilla of different men may vary from 36°·5 (97°·7) to 37°·25 (99°·05). In animals even greater differences are found.[5]

The influence of the temperature of the surroundings.—The temperature of man and other warm-blooded animals is only slightly influenced by the temperature of their surroundings. This fact is well shown by the records of the temperature of men and animals in the tropics and Arctic regions, where the extremes of the temperature of the air occur, in the former +59°C., in the latter −55°C. During a voyage from England to Ceylon, Davy[6] made observations upon the temperatures of seven healthy men under 30 years of age; he found that the average temperature under the tongue was about 36°·9 (98°·4) when the temperature of the air was 15°·6 (60°), and 37°·32 (99°·2) when the air was 26°·4 (79°·5). From these and other observations,[7] he concluded that the temperature of man increases in passing from a temperate into a warm climate, and that the inhabitants of warm climates have a slightly higher temperature than those of mild climates. Reynaud and Blosville[8] found the mean temperature of eight men to be 37°·58 (100°), when under the torrid zone, the temperature of the air varying from 26° to 30° (79°–86°), and 37°·11 (99°) in the temperate zone, with an external temperature varying from 12° to 17° (53°–62°). The average temperature of the mouth was found by Rattray[9] to be 37°·25 (99°) in the tropics, with an external temperature of 25°, as compared with 36°·8 (98°·3), the average temperature in England during the summer heat (18°).

These and further observations, made by Brown-Séquard and others,[10]

[1] "Ueber Krankheiten und Krankheitsverhältnisse auf Island," Schleswig, 1855, S. 24.

[2] *Virchow's Archiv*, 1895, Bd. cxl. S. 125.

[3] Numerous references on this subject will be found in Wunderlich's "Medical Thermometry," *New. Syd. Soc. Translation*, p. 105. See also Bärensprung, *Arch. f. Anat., Physiol., u. wissensch. Med.*, 1851, S. 157; Probyn Williams and Lennard Cutler, *Lancet*, London, 1895, vol. i. p. 932; Giles, *Brit. Med. Journ.*, London, 1894, vol. ii. p. 70. As regards animals, see Hobday, *Veterinary Rec.*, London, 1896, vol. viii. p. 488.

[4] This article, p. 789.

[5] This article, p. 790.

[6] "Researches," London, 1839, vol. i. p. 161.

[7] *Phil. Trans.*, London, 1850, p. 437.

[8] "Animal Heat," article by Edwards in Todd's "Cyclopædia," vol. ii. p. 659.

[9] *Proc. Roy. Soc. London*, 1870, vol. xviii. p. 526.

[10] Brown-Séquard, *Journ. de la physiol. de l'homme*, Paris, 1859, tome ii. p. 152; Gresswell, *Brit. Med. Journ.*, London, 1884, vol. ii. p. 164; Mantegazza, *Presse méd. belge*, Bruxelles, 1863, tome xv. p. 111; Maurel, *Bull. Soc. d'anthrop. de Paris*, 1884, tome vii. p. 371; Jousset, *Arch. de méd. nav.*, Paris, 1883, tome xl. p. 124; Pinkerton, *Journ. Anat. and Physiol.*, London, 1881, vol. xv. p. 118; Edoux and Souleyet, *Compt. rend. Acad. d. sc.*, Paris, 1838 tome vi. p. 456.

show that the effect of tropical heat is to raise the mean temperature of the human body, but the increase is generally less than one degree. Crombie[1] found, as the result of 1288 observations upon himself, that the temperature of the mouth was about 0°·23 higher in Bengal than the average in England, but the difference was greater during the first few weeks of residence in the hot climate.

On the other hand, some observers maintain that residence in a tropical climate does not raise the temperature of the body; thus Boileau[2] states that the normal axillary temperature is between 36°·7 and 37°·2, Thornley[3] and Furnell[4] that it is invariably the same as in England, 36°·9.

Numerous careful observations recently made by Neuhauss[5] during a voyage round the world, show the effect of external heat upon the daily temperature, pulse, and discharge of urine. The following are some of the results:—

Temperature of Air.	Six A.M.	Twelve Noon.	Ten P.M.	Six P.M.	Remarks.
Temperate Zone. Min. 11°·5 | Max. 13°·6	36°·6	36°·9	36°·8	37°·1	Temperature in rectum — Mean of twenty days.
	55	55	56	62	Pulse — Mean of twenty days.
Tropical Zone. 23°·9 | 26°·6	36°·9	37°·3	37°·1	37°·3	Temperature in rectum — Mean of twenty-five days.
	60	68	64	72	Pulse — Mean of twenty-five days.

The influence of the different seasons of the year.—No marked effect upon the heat of the body can be ascribed to the different seasons of the year, apart from that due to variations in external temperature. The numerous observations made by Davy[6] upon himself tend to show that the temperature of the mouth is somewhat lower during the winter months in England, and slightly higher during the summer; a similar series taken in the tropics, in Barbadoes, where the mean annual temperature of the air is 26°·7, and the range throughout the year is about 8°, shows no marked variation during the different seasons.

Jousset[7] found that the cool season caused a fall of two- or three-tenths of a degree in the average temperature of natives of the tropics.

From Bosanquet's[8] observations of the rectal temperature, it appears that the highest sustained average temperature occurred in the winter and early spring months. These determinations were made upon himself four times a day for a period of three years.

A few observations have been made on the influence of winter and summer upon the temperature of animals. Thus Edwards[9] found in the case of sparrows that the mean temperature rose progressively from

[1] *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 550.
[2] *Lancet*, London, 1878, vol. i. p. 413.
[3] *Ibid.*, 1878, vol. i. p. 554.
[4] *Ibid.*, 1878, vol. ii. p. 110.
[5] *Virchow's Archiv*, 1893, Bd. cxxxiv. S. 365.
[6] *Phil. Trans.*, London, 1845, pt. 2, p. 319; and 1850, p. 437.
[7] *Arch. de méd. nav.*, Paris, 1883, tome xl. p. 124.
[8] *Lancet*, London, 1895, vol. i. p. 672.
[9] "Animal Heat," in Todd's "Cyclopædia," vol. ii. p. 659.

the depth of winter to the height of summer; in the month of February the mean temperature was 40°·8, in April 42°, and in July 43°·77; from this time the temperature began to decline. It was also found that, in winter, birds could more readily resist the action of extreme cold than in summer.

Davy[1] observed the temperature of sheep during summer and winter, and his results, although they are not sufficiently consistent for positive conclusions, seem to show that the temperature of the body is a little higher in the warm weather than in the cold.

The influence of extreme heat and cold.—The experience of the inhabitants of tropical climates shows that it is possible to live even in an atmosphere the temperature of which at times exceeds that of the body, and that the body is able, by means of the cooling effect of the evaporation of sweat, to prevent its temperature rising a degree above the normal.

Lining,[2] in 1738, found that the temperature of his axilla was 36°·1, and that of his mouth 36°·7, when the heat in the sun's rays was 51°·1, in the shade 36°·7, on a hot summer's day in South Carolina. Ellis,[3] in 1758, observed that the temperature of his body was not above 36°·1 when he was living in Georgia, and the temperature of the air was 40°·6. Experiments on men and on lower animals have shown that much greater heat can be borne for short periods. Blagden and Fordyce[4] observed their own temperatures after remaining in heated rooms, and found that the effect varied according to the amount of moisture present; thus, after remaining fifteen minutes in a damp room heated to 54°·4, the temperature of the mouth and urine was 37°·8, but a similar exposure in a dry room heated to 115°·5—126°·7, and in which beefsteaks were being cooked by the heat of the air, did not raise the temperature of the body above the normal. Similar experiments were made by Dobson,[5] who found that the temperature in the mouth of one man rose to 37°·5 after he had remained about fifteen minutes in a room heated to 94°·4; in another case the rise was to 38°·6, after twenty minutes' exposure to air at 98°·9; and in a third case a stay of ten minutes in a room at 106°·7 caused a rise to 38°·9.

Tillet[6] had previously observed young girls remain without any inconvenience for five or ten minutes in a kiln heated to about 130,° but he does not give any records of their temperature. In 1747, Le Monnier[7] found that he could remain for eight minutes in a bath supplied by a thermal spring, the temperature of which was 44° to 45°; at the end of that time his skin was red and swollen, and his distress so great that he was obliged to get out. No observations upon the temperature of the body are given. Kurrer[8] and Neuhauss[9] have observed that the temperature of stokers, working in a stoke-hole at 50° to 56°, is raised to 37°·6, or even to 38°·1.[10]

Numerous experiments have been made to determine the effect of

[1] "Researches," London, 1839, vol. i. p. 208.
[2] *Phil. Trans.*, London, 1748, vol. xlv. p. 338.
[3] *Ibid.*, 1758, vol. l. pt. 2, p. 754.
[4] *Ibid.*, 1775, vol. lxv. pt. 1, pp. 111 and 484.
[5] *Ibid.*, 1775, vol. lxv. pt. 2, p. 463.
[6] *Hist. Acad. roy. d. sc.*, Paris, 1764, p. 188.
[7] *Ibid.*, 1747, p. 271.
[8] *Deutsche Vrtljschr. f. öff. Gsndhtspflg.*, Braunschweig, (2), Bd. xxiv. S. 291.
[9] *Virchow's Archiv*, 1893, Bd. cxxxiv. S. 365.
[10] See also Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 601.

extreme heat upon animals. Provoost and Fahrenheit, working under the direction of Boerhaave,[1] found that a dog and a cat placed in a hot stove (63°) died in twenty-eight minutes, whilst a sparrow, under similar conditions, died in seven minutes. Duntze[2] observed that dogs could live in an atmosphere at 42°·2, but died when the temperature was raised to 45°. It was found by Delaroche[3] that cats, rabbits, pigeons, and various insects could remain for one hour in a temperature of 36° without fatal results; the most marked symptom was the greatly quickened respiration. When the temperature was raised to 45° or 53°, the cat and rabbit died within two hours, the pigeon in one hour and twenty minutes, the most marked symptom being convulsions. A frog, under similar conditions, was alive at the end of two hours. The temperature of a rabbit exposed to a heat of 45° for one hour and forty minutes rose from 39°·7 to 43°·8. Exposure to moist heat quickly raised the temperature of animals, as shown in the following table:—

Animal.	Temperature before.	Temperature after.	Moist Heat.	Time of Exposure.
Rabbit	39°·6	43°	38°·7	55 minutes
Guinea-pig	38°·4	44°·2	40°·7	55 ,,
Pigeon	41°·8	46°·9	41°·9	42 ,,
Frog	Not stated	26°	25°·6	73 ,,
,,	,,	27°·8	27°·2	50 ,,

The effect of dry and moist hot air upon different animals was determined by Bernard[4] in numerous experiments; some of the results are here given:—

Animal.	Dry Air.	Death.	Animal.	Dry Air.	Death.
Pigeon	90°	In 6 minutes	Rabbit	100°	In 10 minutes
,,	90°	6½ ,,	,,	80°	18 ,,
Dog	90°	24 ,,	,,	80°	17 ,,
Guinea-pig	100°	5 ,,	,,	65°	25 ,,
,,	100°	6 ,,	Dog	100°	18 ,,
Rabbit	100°	10 ,,	,,	90°	24 ,,
Dog	100°	18 ,,	,,	80°	30 ,,
Rabbit	100°	7 ,,			

In moist hot air the animals died very quickly; thus, when the temperature was 80°, 60°, and 45°, the rabbits died in two, three, and ten minutes respectively. Experiments made by immersing the body of the animal in hot water gave similar results. To determine the effect of exposing the body to dry heat without warming the air used for respiration, Bernard made the following comparative experiments upon rabbits of similar size:—

[1] "Praelect. Anat.," p. 211; "Elém. de chymie," tome i. pp. 148, 277, 278.
[2] Quoted from Delaroche ([3]).
[3] *Journ. de phys.*, Paris, 1806, tome lxiii. pp. 207, 468; 1810, tome lxxi. p. 289.
[4] *Gaz. méd. de Paris*, 1859, tome xiv. p. 462; "Leçons sur la chaleur animale," 1876, p. 349.

(*a*) Rabbit placed in dry air 100°—

Temperature before = 40°
„ after 5 minutes = 41°
„ „ 10 „ = 44°—respiration quickened.
„ „ 16 „ = 44°·5—death.

(*b*) Head of rabbit placed in dry air 100°, body in cool air—

Temperature before = 40°
„ after 5 minutes = 40°
„ „ 10 „ = 40°—respiration quickened.
„ „ 15 „ = 41° } respiration very rapid.
„ „ 20 „ = 41° }
„ „ 25 „ = 43° }
„ „ 30 „ = 43° }
„ „ 38 „ = 43°—death.

(*c*) Body of rabbit in dry air 100°, head in cool air—

Temperature before = 39°·5
„ after 4 minutes = 42°
„ „ 10 „ = 43°—respiration quickened.
„ „ 15 „ = 44°
„ „ 20 „ = 45°—death.

Obernier[1] found that when the external temperature was first raised the rectal temperature of dogs and rabbits fell slightly, about 0°·4, but soon after the air reached 30° to 35° the temperature of the animal began to rise. Death generally resulted before the internal temperature rose to 45°, but in one case it reached 46°·2. The most important symptoms were restlessness, quickening of respiration and pulse, and finally convulsions and loss of consciousness. A short time before death it was impossible to feel the pulse, a fact explained by the fibrillar contraction of the heart observed by Obernier when the thorax was opened. An examination of the body directly after death showed marked congestion of the brain and lungs; the muscles were inexcitable, and quickly went into rigor mortis. Similar changes were observed in the bodies of soldiers who had died from sunstroke.

Numerous facts show that cold-blooded animals can live in hot media. Thus, internal parasites of mammals and birds can live in surroundings at temperatures of 37° and 43°·9; and there are well-authenticated cases of fishes living in springs as hot as 37°–44°.[2] Sonnerat[3] even states that he saw fish actively swimming about in the hot water (60°–62°) of thermal springs in New Guinea ; it is doubtful, however, if the temperature was correctly recorded in this case.

It has been shown by Davenport and Castle[4] that by gradually raising the temperature tadpoles can be kept alive in warm water. Hertwig[5] has observed that no development takes place in the ova of the frog when the temperature of the water is zero, but between 2° and 33° it progresses with different rapidity, cold delaying, warmth hastening the process. A temperature, however, of 34° is fatal.

[1] "Der Hitzschlag," Bonn, 1867.

[2] Spallanzani, "Opusc. de phys. anim.," tome i. pp. 54–69, 101 ; Desfontaines, quoted from Gavarret, "De la chaleur produite pas les êtres vivants," Paris, 1855, p. 464 ; Tripier, *Compt. rend. Acad. d. sc.*, Paris, tome ix. p. 602 ; Cumberland, *Biblioth. univ.*, Genève, 1839, tome xx. p. 204 ; Prinsep, *ibid.*

[3] "Voyage a la Nouvelle Guinée," Paris, 1776, pp. 38–41.

[4] *Arch. f. Anat. u. Entwcklngsgesch.*, Leipzig, 1885, Bd. ii. S. 227.

[5] *Sitzungsb. d. preuss. Akad. d. Wissensch.*, 1896, S. 105.

Numerous observations show that the temperature of animals living in the Arctic regions is equal to that of animals of the same classes in temperate climates. The following are some of the results obtained by different explorers :—

Animal.	Temperature of Animal.	Temperature of Air.	Observer.
Arctic fox	38°·3	−35°·6	Parry and Lyon.[1]
	41°·1	−35°·6	,,
	39°·4	−32°·8	,,
Wolf	40°·5	−32°·8	,,
White hare	38°·3	−29°·4	,,
Prairie fowl (male)	43°·2	−12°·7	Black.[2]
	43°·0	−15°·0	,,
Prairie fowl (female)	42°·8	−8°·3	,,
	43°·3	−8°·0	,,
	42°·8	−1°·1	,,
Willow grouse (male)	42°·4	−19°·7	,,
	43°·3	−32°·8	,,
	43°·3	−35°·8	,,

The limits of extreme cold are generally reached when the water in which the animals live, or the lymph of their tissues, is frozen. Fishes live in salt water when the temperature is below zero, but usually die when the water is frozen.

Boyle[3] exposed lampreys in a vessel of water to an exceedingly sharp frost, and found next day that one lamprey was frozen in the ice; when the ice was partly broken and partly thawed the animal was at first motionless, but in a few minutes recovered, and dragged after it a large piece of ice in which its tail was fixed. Similar experiments were made with similar results upon gudgeons and frogs. Hunter[4] found by experiment that the internal temperature of a frog and an eel could be reduced to −0°·6, and that, although the animals appeared to be dead, they revived when the temperature rose. Regnard[5] found that carp will live in water containing $2\frac{1}{2}$ per cent. of magnesium sulphate, even when the temperature is a degree or two below zero; at −2° the fish appear to be asleep, and at −3° their vitality is so greatly reduced that they seem to be dead, but revive when the water is gradually warmed. Pictet[6] exhibited at one of his lectures frozen gold fish, pike, and frogs, and at the next lecture the same animals alive and well after gradual thawing. According to this observer, fishes can be rapidly frozen so hard that they can be snapped in two, and yet other fishes frozen equally hard recover when slowly thawed. It has been observed by Marcet[6] that gold fish completely embedded in the ice showed no signs of life on thawing, but one fish, which was partly encased in ice and was surrounded by a little water, appeared lifeless, but recovered perfectly in a short time. Observations and experiments made by

[1] Parry, "Journal of a Second Voyage for the Discovery of a North-West Passage," London, 1824, p. 157; *Ann. de chim. et phys.*, Paris, 1825, Sér. 2, tome xxviii. p. 223.

[2] *Compt. rend. Acad. d. sc.*, Paris, 1836, tome ii. p. 621.

[3] "Philosophical Works," Shaw's edition, vol. i. p. 688.

[4] "Works," Palmer's edition, London, 1837, vol. iv. p. 131 *et seq.*

[5] *Compt. rend. Soc. de biol.*, Paris, 1895, p. 652.

[6] Quoted from Marcet, Croonian Lectures, *Brit. Med. Journ.*, London, 1895, vol. i. p. 1367.

Gaymard[1] and Gavarret[2] show that toads and fishes may be frozen perfectly stiff and yet revive when gradually thawed; according to the former observer, the freezing must be gradual, otherwise the animals are killed. During Franklin's[3] explorations in the Arctic regions, it was observed that fish frozen completely hard recovered when they were thawed; a carp, which had been frozen for thirty-six hours, was able after it was thawed to leap about with much vigour.

The influence of baths.—A warm or cold bath has a greater effect upon the temperature of the body than exposure to air at the same temperature, for the power of conduction of water is greater than that of air. The first important experiments upon this subject were made by Currie in 1797.[4] He found that the immediate effect of a cold bath might be a slight rise in the temperature of the mouth, but the permanent effect was a fall. The following are some of his results:—

Temperature of Bath.	Duration of Bath.	Temperature before the Bath.	Temperature after the Bath.
Sea water 6°·7	12 minutes	36°·7	34°·0
,, 5°·7	30 ,,	36°·3	34°·3
Fresh water 4°·3	34 ,,	36°·7	33°·7

The temperature was taken in the mouth, and therefore the depression was greater than it would have been in the rectum.

Fleury[5] found the temperature in the mouth sink to 34°, 32°·9, and even to 29° during a cold bath; Virchow[6] observed a fall to 34°; Speck[7] found that the immediate effect of a shower bath at 22° was to raise the temperature of the mouth, but after ten minutes' exposure the temperature fell 1°·23.

Numerous observations have been made by Liebermeister,[8] who selected the temperature of the closed axilla as representing more exactly the temperature of the body. He concludes that the immediate effect of a cold bath is to slightly raise the temperature, and that a bath of moderate cold and duration does not lower the temperature below the normal, for an increase in the heat production compensates for the increased loss. Liebermeister, as Currie had previously done, used the bath as a water calorimeter, and calculated that in a bath of from 20° to 30° the heat production was three or four times greater than the normal. Jürgensen[9] confirmed many of these results; he found that the rectal temperature of men did not fall more than 1°, often less, after remaining twenty-five minutes in a cold bath at 11° to 9°. Recently Lefèvre[10] has given excellent proofs of the power of regulation of

[1] *Biblioth. univ.*, Genève, 1840, tome xxvi. p. 207.
[2] "De la chaleur produite par les êtres vivants," Paris, 1855, p. 502.
[3] Franklin, "Journey to the Polar Sea," 1819–1822, 2nd edition, vol. ii. p. 17.
[4] "Medical Reports on the Effect of Water, Cold and Warm, as a Remedy in Fever and other Diseases."
[5] *Progrès med.*, Paris, 1858, p. 337. [6] *Virchow's Archiv*, 1858, Bd. xv. S. 70.
[7] *Arch. d. Ver. f. gemeinsch. Arb. z. Förd. d. wissensch. Heilk.*, Göttingen, 1861, Bd. v. S. 422.
[8] *Arch. f. Anat. Physiol. u. wissensch. Med.*, Leipzig, 1860, S. 520, 589; "Handbuch d. Path. u. Therap. des Fiebers," 1875, S. 102.
[9] *Deutsches Arch. f. klin. Med.*, Leipzig, 1867, Bd. iii. S. 165; Bd. iv. S. 110, 323.
[10] *Compt. rend. Soc. de biol.*, Paris, 1895, p. 559; 1896, pp. 492, 564.

temperature in man. He remained three hours in a bath at 15°, and yet his axillary temperature fell only one degree (37°·30 to 36°·30 in the first two hours and a half, and then remained stationary at 36°·30); the amount of heat lost was 800 kilo-calories. A bath in water at 25° for three hours caused a fall in temperature from 37°·20 to 36°·60, with a loss of 312 kilo-calories; while a bath of one hour's duration in water at 7° caused a fall from 37°·70 to 36°, the loss of heat being 530 kilo-calories.

In comparing the effect of baths on different people, it is important to consider the size of the body and the amount of subcutaneous fat, for the greater the size and amount of fat the slower is the cooling of the body. Liebermeister found that the temperature of the axilla of a fat man only fell 0°·2 during a bath of 21° to 30°, lasting one hour and a half.

The effect of a warm bath is to raise the temperature, but after the bath there is, as Currie and Liebermeister observed, a fall in temperature followed by a gradual rise to the normal.

It is impossible here to consider all the numerous results, some contradictory, which have been obtained by different observers.[1] It is important, however, to note that the different results markedly show the power of compensation possessed by the higher animals. A cold bath abstracts a large quantity of heat, but within certain limits does not cause the temperature of the body to fall, for the cutaneous blood vessels contract and thus diminish the loss of heat, and the cold acting on the nervous system stimulates the tissues to increased production of heat; on the other hand, a hot bath would quickly cause a rise in temperature, if the animal were not able within certain limits to increase its loss of heat by an excessive vascularity of the skin and to diminish its production of heat. These compensating factors show their influence by a rise in temperature after a cold bath and by a fall after a hot bath, as the case may be. For this reason a hot bath is most effective in producing a cooling effect upon the body in tropical climates. The after-effects, however, soon disappear, and the temperature becomes normal.

The compensation is, in fact, so exact in a healthy man, that any fall or rise in temperature, caused by too long exposure to cold or heat, is followed respectively by a rise above or fall below the normal. Thus it is that the mean daily temperature and the daily variations are very slightly or not at all affected by baths (Jürgensen,[2] Liebermeister,[3] Ringer and Stuart,[4] and others). Still it must be remembered that this compensation is only effective within certain narrow limits,[5] and does not in any way invalidate the use of cold baths in the treatment of high temperatures in cases of fever.

Experiments upon the influence of warm and cold baths have also been made upon animals, and the results agree with those obtained upon man. Crawford[6] in 1871 found that the temperature of a dog kept in a hot bath, 45°·6 to 44°·4, rose in thirty minutes to 42°·8, and

[1] For further details and references see Liebermeister, "Handbuch d. Path. u. Therap. des Fiebers," Leipzig, 1875; Wunderlich, "Medical Thermometry," p. 109.

[2] *Loc. cit.* [3] *Loc. cit.*

[4] *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 203.

[5] Löwy, *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xlv. S. 625; 1890, Bd. xlvi. S. 189; see also "Chemistry of Respiration," this Text-book, vol. i. p. 712.

[6] *Phil. Trans.*, London, 1781, vol. lxxi. p. 486.

the dog became very languid; the venous blood of dogs kept in a warm bath had an arterial colour, whereas a cold bath, 7°·2, rendered the blood in the jugular vein very dark. More extended observations were made by Hoppe[1] upon both the immediate and after effects of baths upon dogs. The rectal temperature of a dog placed in water at 48° for three minutes rose from 38°·75 to 41°·45; a cold bath at 9°·12, lasting half a minute, caused a fall of 1°; a bath of freezing water, lasting respectively two and four minutes, produced a fall of 1°·7 and 4°·88 below the normal. Hoppe found that the temperature fell during a cold bath but afterwards rose above the normal, that it rose during a hot bath but afterwards fell below the normal. The sensation of cold stimulated the organism to an increased production of heat, for if evaporation from the wet skin was rapid the temperature rose, but if it was hindered by a covering of rubber the temperature fell.

Bernard[2] found that very hot baths quickly caused death, the symptoms being similar to those observed from exposure to hot air.

The influence of certain drugs upon the temperature of the body.—*Alcohol.*[3]—The effect of alcohol is a fall in temperature, and not, as is popularly believed, an increased heat of the body. It is true that after the use of alcohol there is a feeling of increased warmth, but this is due only to the increased vascularity of the skin and the activity of the sweat glands.

Alcohol seems to act in two ways: it has little or no effect upon the production of heat in the tissues, but greatly increases the loss of heat by causing the cutaneous vessels to dilate, stimulating the sweat glands and quickening the circulation. The normal reaction to cold, namely, increased production of heat and contraction of the cutaneous vessels, is partly paralysed by large doses of alcohol, with the result that drunkards exposed to cold quickly "freeze" to death.

Various observers[4] have found that alcohol taken in ordinary quantities as a beverage causes a slight depression, generally less than half a degree, in the temperature of healthy men; on the other hand, poisonous doses may cause a fall of five or six degrees—in fact, many of the lowest temperatures recorded in man have been observed in drunken persons exposed to cold.

Experiments upon animals have given similar results. Walther[5] exposed two rabbits to a temperature of 21°·2 below zero; in two and a quarter hours the temperature of the normal rabbit fell from 38°·8 to 35°·6, while that of the rabbit which had received 35 c.c. of brandy fell from 38°·8 to 19°·8. A guinea-pig was given a dose of 6 or 7 grms. of brandy, and then exposed to moderate cold; its temperature fell 10°,

[1] *Virchow's Archiv*, 1857, Bd. xi. S. 453.

[2] This article, p. 815.

[3] For further details, see works on therapeutics.

[4] Davy, *Phil. Trans.*, London, 1850, p. 444; Lichtenfels and Fröhlich, *Denkschriften d. k. Akad. d. Wissensch.*, Wien, 1852, Bd. iii. Abth. 2, S. 131; Lallemand, Perrin, and Duroy, "Du rôle de l'alcool et des anesthésiques dans l'organisme," Paris, 1860; Ogle, *St. George's Hosp. Rep.*, London, 1866, vol. i. p. 233. Ringer and Rickards, *Lancet*, London, 1866, vol. ii. p. 208; Cuny Bouvier, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 370; Godfrin, "De l'alcool, son action physiologique, ses applications thérapeutiques," 1869; Weckerling, *Deutsches Arch. f. klin. Med.*, Leipzig, 1877, Bd. xix. S. 317; Zuntz, *Fortschr. d. Med.*, Berlin, 1887; Geppert, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. xxii. Parkes and Wollowicz, *Proc. Roy. Soc. London*, 1870, vol. xviii. p. 362, found that alcohol in ordinary quantities had no effect on the temperature of a healthy man.

[5] *Arch. f. Anat., Physiol. u. wissensch. Med.*, Leipzig, 1865, S. 45.

whereas that of a normal animal exposed to cold only varied one, or two-tenths of a degree. Similar results have been obtained by others.[1]

Calorimetric observations have been made by Reichert[2] upon the influence of alcohol on the production and loss of heat in dogs; he found that the total heat production was not essentially altered, but the loss exceeded the production, and therefore the temperature fell. The doses given were 1·25, 2·5, and 5 c.c. per kilo. of the animal's weight.

Chloroform, ether, morphia, chloral, and nicotine.—The general effect of these drugs is to cause a fall in the temperature of the body,[3] and in poisonous doses to so greatly depress the power of heat regulation that a warm-blooded animal passes into a condition in which it cannot maintain its temperature, its respiratory exchange and temperature varying with, and in the same direction as, that of its surroundings (Rumpf, Pembrey). Calorimetric observations made by J. Rosenthal show that under the influence of chloral the temperature of rabbits falls,[4] the discharge of heat is 30 to 40 per cent. greater than the normal, and the production of heat and also of carbon dioxide is diminished; strychnia and tetanus, on the other hand, increase the production but diminish the loss of heat.

Cocain,[5] atropin, brucin, caffein, and veratrin raise the temperature; the most remarkable pyretic drug, however, is β-tetra hydronaphthylamine, which causes in the case of rabbits a rapid rise of three or four degrees in the rectal temperature[6]; curari[7] causes a marked fall in temperature.

The limits of bodily temperature compatible with life.—Although the range of temperature in a normal man is less than 2°, yet a much wider range is observed in certain pathological conditions. Thus by exposure to cold, especially when the subjects are drunk, the temperature may fall even as low as 24° without a fatal issue. Reincke[8] has recorded numerous cases of low temperature resulting from the accidental exposure of drunkards to cold air and water. In two of these cases the rectal temperature was 30° and 24° respectively; the patients were unconscious, but under treatment

[1] Rumpf, *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiii. S. 538; Ringer and Rickards, *loc. cit.*; Tscheschichin, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1866, S. 161; Cuny Bouvier, *loc. cit.*

[2] *Therap. Gaz.*, Detroit, February, 1890.

[3] Dumeril and Demarquay, "Recherches expérimentales sur les modifications imprimées à la temperature animale par l'ether et l'chloroforme," 1848; Brown-Séquard, *Compt. rend. Soc. de biol.*, Paris, 1849, No. 7, p. 102; Tscheschichin, *loc. cit.*; Lallemand, Perrin, and Duroy, "Du rôle de l'alcool et des anesthésiques dans l'organisme," Paris, 1860; Spencer Wells, *Edin. Med. Journ.*, 1869, 1870; Richardson, *Practitioner*, London, 1869, 1870; Waren Tay, *Brit. Med. Journ.*, London, 1870, vol. i. p. 329; Oglesby, *Practitioner*, London, 1870; Angelesco, *Compt. rend. Soc. de biol.*, Paris, 1894, p. 786; Richet, *Compt. rend. Acad. d. sc.*, Paris, 1889, tome cix. p. 190; *Arch. de physiol. norm. et path.*, Paris, 1890, tome ii. p. 221; Warter, *Med. Times and Gaz.*, London, 1866, vol. ii. p. 416; Lichtenfels and Fröhlich, *Denkschriften d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, 1852, Bd. iii. Abth. 2, S. 137; Hobday, *Journ. Comp. Path. and Therap.*, Edin. and London, vol. viii. p. 287; Pembrey, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894–1895, vol. xvii.

[4] *Sitzungsb. d. k. Akad. d. Wissensch. zu Berlin*, 1890, Bd. xx.; xxi. p. 393.

[5] Mantegazza, *Ann. univ. di med. e chir.*, Milano, 1859, vol. clxvii.; U. Mosso, *Arch. ital. de biol.*, Turin, 1887, vol. viii. p. 370; 1891, vol. xiv. p. 288; Hobday, *Journ. Comp. Path. and Therap.*, Edin. and London, 1895, vol. viii. p. 20; 1897, vol. x. p. 80.

[6] Stern, *Virchow's Archiv*, 1889, Bd. cxv. S. 14; Fawcett and Hale White, *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 435.

[7] This article, p. 841.

[8] *Deutsches Arch. f. klin. Med.*, Leipzig, 1875, Bd. xvi. S. 12.

recovered in a day or two. In other cases, with temperatures 28°·4, 27°, and 26°·4, death followed in about twenty-four hours. In a case observed by Nicolaysen [1] the rectal temperature was 24°·7, but the drunkard, who had been exposed for a whole night to air 6° below zero, completely recovered; the temperature of the vagina and axilla was 27°·9 in a woman who had had a similar experience, but within six hours the temperature rose to 36°·3 under treatment, and the patient completely recovered.[2] In four cases of insanity, Löwenhardt [3] has observed temperatures as low as 25°, 29°·5, 23°·75, and 28°; in one case the range of temperature for several weeks was from 25° to 35°. The patients were about 60 years of age; they often ran about naked in cold weather, and were frequently bathed on account of their dirty habits, and although they were fairly active they did not take much food. The observations were taken sometimes in the axilla, sometimes in the rectum.

Weiland [4] has recorded two cases of adults with temperatures reduced to 28°·4 and 26°·6 from exposure to cold; the observations were taken in the rectum several hours before death; in a third case, that of a drunkard who had been exposed to cold, the rectal temperature was 30°·4, and recovery took place. The rectal temperature of a man suffering from bronchi-ectasis was found by Liebermeister [5] to be 32°·6, and that of a child five days old, suffering from sclerema and icterus, 32°·15; the readings were taken a day or two before death, and several thermometers were used and tested. Köhler [6] observed a temperature of 28°·2 in the rectum of a drunkard, and found that, notwithstanding treatment, it remained low until shortly before the man's death a month later; two cases, with rectal temperatures 26°·8 and 26°·7, were observed by Quincke;[7] the subnormal temperature was due to exposure to cold, but both of the patients recovered. Numerous records of subnormal temperatures will be found in papers by Janssen,[7] Lemcke,[8] and Glaser.[9]

In the case of non-hibernating mammals an artificial cooling of the body to 18° is in a few hours followed by death, unless artificial respiration and heat be applied. Rabbits cooled to 18° are perfectly helpless and paralysed; the heart-beat is feeble, 16 to 20 per minute; the respiration is either exceedingly slow or rapid and shallow; the nerves and muscles long remain irritable, and during operative procedures there is very little bleeding, owing to the low blood pressure.[10]

It was shown by Edwards [11] that newly-born pups and kittens would live for two or three days with their temperature reduced as low as 17° or 20°, and that the application of artificial warmth would restore the young animals, if this low temperature had not persisted too long. Adult animals, however, when cooled to 18° or 20°, generally died, even

[1] *Jahresb. ü. d. Leistung. . . . d. ges. Med.*, Berlin, 1875, Bd. i. S. 283.
[2] Peter, *Gaz. hebd. de méd.*, Paris, 1872, p. 499.
[3] *Allg. Ztschr. f. Psychiat., etc.*, Berlin, 1868, Bd. xxv. S. 685.
[4] *Schrift. d. Univ. zu Kiel*, 1869, Bd. xvi.
[5] "Handbuch d. Path. u. Therap. des Fiebers," 1875, S. 69.
[6] *Schrift. d. Univ. zu Kiel*, 1873, Bd. xx.
[7] Quoted from Janssen, *Deutsches Arch. f. klin. Med.*, Leipzig, 1894, Bd. liii. S. 249.
[8] *Ibid.*, 1883–84, Bd. xxxiv. S. 90.
[9] "Ueber Vorkommen und Ursachen abnorm niedriger Körpertemperatur," Diss., Bern, 1878.
[10] Walther, *Virchow's Archiv*, 1862, Bd. xxv. S. 414; *ibid.*, 1865, S. 25; Horvath, *Verhandl. d. phys.-med. Gesellsch. in Würzburg*, 1881, Bd. xv. S. 187; Tscheschichin, *Arch. f. Anat., Physiol. u. wissensch. Med.* 1866, S. 151.
[11] "De l'influence des agens physiques sur la vie," 1824, p. 237.

when artificial warmth was applied. Similar results were obtained in the case of recently hatched and old birds.

Hibernating mammals have been observed during winter with temperatures as low as 2°, and during summer they may be cooled by artificial means to 1°·2; in these cases the animals are able to again raise their temperature without any external aid (Walther, Horvath, and others).

The eggs of silk-worms and of other insects may be exposed for a long time to temperatures 20° to 30° below zero, and yet will develop into larvæ when removed to warm surroundings.[1] The Arctic explorer Ross exposed caterpillars to a temperature of –42°, and found that they recovered when slowly thawed. Colasanti[2] observed that hens' eggs could be exposed for two hours to a temperature of –4°, and for half an hour to a temperature of –7° to –10°, and yet developed normally when placed in an incubator.

As already pointed ont on p. 817, in the lower vertebrates the temperature of the body may sink to zero and yet recovery take place. Hunter[3] placed an eel in a freezing mixture, until the temperature of its stomach fell to –0°·6, when the animal appeared to be dead, but by the next day it had recovered; a similar result was observed in a frog. Frozen leeches, however, were dead when thawed.

As regards the limit of high temperatures compatible with human life, there are numerous records of cases of hyperpyrexia. The highest observed by Wunderlich[4] was 44°·75 (112°·55 F.) in a case of tetanus; one hour after death the temperature was 45°·37. Currie[5] found a temperature of 44°·45, Woodman[6] one of 46·1 in fatal cases of scarlet fever; Bäumler[7] records a case of sunstroke in a healthy man, the temperature in the axilla was 42°·9, there was deep coma, and death took place in eight hours; in a similar case observed by Casey[8] the temperature in the axilla was 43°·1, and death occurred within three hours. Levick[9] gives cases of sunstroke in which the temperature was 42°·8, and the patients recovered. Fatal cases with temperatures 43°, 42°·5, and 44° are recorded by Simon,[10] two cases of tetanus with temperatures 44°·4 and 41°·6 before death by Lehmann,[11] and others with 43°·4, 43°·6, 42°·75, 43°·4, 43°·4, 44°·3, and 43° by Quincke.[12]

On the other hand, Donkin[13] gives cases of temperatures as high as 44°·2, 45°, and 44°·5, in which recovery took place; the high temperature, however, appears to have persisted for a very short time. In two cases of rheumatic hyperpyrexia recorded by Arkle[14] the temperature was 43°·55 (110°·4 F.), but the patients recovered.

[1] Réaumur, "Mém. sur les insectes," tomes ii. and v.; Spallanzani, "Opusc. de phys. anim.," tome i. pp. 82–85; Bonafous, "*Biblioth. univ.*, Genève, 1838, tome xvii. p. 200; Ross, *ibid.*, 1836, tome iii. p. 423; Pictet, *Arch. d. sc. phys. et nat.*, Genève, 1893 (3), tome xxx. p. 293.

[2] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1875, S. 477.

[3] "Works," Palmer's edition, London, 1837, vol. iv. p. 131 *et seq.*

[4] "Medical Thermometry," p. 204.

[5] "Medical Reports, etc."

[6] *Med. Mirror*, London, 1865, p. 77.

[7] *Med. Times and Gaz.*, London, 1868, vol. ii. p. 118.

[8] *Ibid.*, 1866, vol. ii. p. 26.

[9] *Penn. Hosp. Rep.*, Philadelphia, 1868, vol. i. p. 369.

[10] *Charité-Ann.*, Berlin, 1865, Bd. xiii. Heft 2, S. 1.

[11] *Schmidt's Jahrb.*, Leipzig, 1868, Bd. cxxxix. S. 241.

[12] *Berl. klin. Wchnschr.*, 1869, S. 301.

[13] *Brit. Med. Journ.*, London, 1879, vol. ii. p. 983.

[14] *Trans. Clin. Soc. London*, 1888, vol. xxi. p. 187.

Richet[1] has collected three cases in which the temperature rose to 46°, but the patients recovered. Numerous other cases of high temperature in man are to be found scattered throughout medical literature.[2]

Experiments upon animals have determined more exactly the limit of high temperature. Bernard[3] found that when the internal temperature of rabbits was artificially raised to 45° they died; in birds the fatal limit was 51° or 52°. According to this physiologist, death was due to stoppage of the heart by the hot blood, which sent the muscle into rigor mortis. Rosenthal[4] obtained similar results for rabbits, but found that if the animal was removed to cooler surroundings when its temperature had reached 44°, recovery might take place. From these and similar experiments by Obernier,[5] Wood,[6] and others, it may be concluded that a bodily temperature of 45° is extremely dangerous, and one of 47° quickly fatal, to the life of mammals. The limit of high temperatures appears to be fixed by the point at which the proteids of the body begin to coagulate.

The Temperature of Different Parts of the Body.

The heat of the body is produced by processes of combustion taking place chiefly in the muscles and glands, while heat is lost chiefly from the surface of the skin. The result, therefore, is that the temperature of the body diminishes from the interior to the surface. It is impossible, however, to give any exact value to the temperature of different parts, because the production and loss of heat vary under different conditions of the animal, such as muscular activity and digestion.

The temperature of internal parts in man.—In considering this subject, it is important to remember that the temperature taken by a thermometer placed in a dry, well-closed axilla represents the heat of an internal cavity; Ringer and Stuart[7] even state that, "due care being taken and sufficient time allowed, the temperature of the axilla is always identical with that of the mouth, and with that of the rectum four to six inches above its termination."

Upon the respective temperatures of the mouth, axilla, and rectum, there is a great want of agreement among observers. This is in great part due to the fact that in numerous cases insufficient time is allowed for the determination of temperature in the mouth and axilla; but there is another cause, which is beyond the control of the observer—the circulation of blood in the mouth and in the skin of the axilla is liable to marked variations. It will be well, therefore, to mention the discordant results obtained, and then draw some general conclusion. As just mentioned, Ringer and Stuart state that the temperature in the axilla is identical with that of the mouth and rectum; Ogle[8] says that

[1] *Compt. rend. Soc. de biol.*, Paris, 1894, p. 416.

[2] Hale White, *Brit. Med. Journ.*, London, 1894, vol. ii. p. 1093. Here numerous references will be found. See also *Trans. Clin. Soc. London*, 1882, vol. xv. p. 261.

[3] *Gaz. méd. de Paris*, 1859, tome xiv. p. 462; "Leçons sur la chaleur animale," p. 349.

[4] "Zur Kenntniss der Wärmeregulirung bei den warmblütigen Thieren," Erlangen, 1872, S. 15.

[5] "Der Hitzschlag," Bonn, 1867, S. 71.

[6] "Fever," *Smithson. Contrib. Knowl.*, Washington, 1880, No. 357.

[7] *Proc. Roy. Soc. London*, 1877, vol. xxvi. p. 186.

[8] *St. George's Hosp. Rep.*, London, 1866, vol. i. p. 233.

if the thermometer be warmed by the hand and then kept under the tongue in the closed mouth for eight minutes, the reading is the same as that obtained by inserting the thermometer in the urine as it leaves the body. On the other hand, Crombie[1] found, as the result of comparative experiments in which care was taken to obtain accurate results, that in fifteen simultaneous observations the mean difference of temperature in the mouth was 0·13 above the reading in the axilla, and in thirty-five determinations the mean difference in the rectum was 0·22 above the temperature in the mouth. A number of simultaneous observations made by Parkes and Wollowicz[2] show that the rectal temperature of a healthy man may be 0·3 to 0·6 higher than the temperature in the axilla. Gassot[3] made comparative observations at different times of the day, with the following results :—

Time.	Man.		Woman.		
	Mouth.	Axilla.	Mouth.	Axilla.	Rectum.
7 a.m.	37°·06	37°·78	37°·0	36°·8	37°·7
2 p.m.	37°·6	37°·3	37°·6	37°·3	38°·0
9 p.m.	37°·4	37°·12	37°·5	37°·3	37°·8

Oertmann[4] observed, when the thermometer was kept in the axilla for fifteen minutes, in the rectum at a depth of 7 cm. for five minutes, and in the stream of urine for five seconds, that the temperature of the urine was generally equal to that of the rectum, but four-tenths of a degree higher than that of the axilla. Ten simultaneous observations of the temperature in the mouth and rectum gave an average difference of 0·32 in favour of the latter (Cuny Bouvier).[5] According to Liebermeister,[6] the rectal temperature is 0°·1 to 0°·4 above that of the axilla.

Lorain[7] maintained that the temperature in the rectum or vagina alone represented the internal temperature of the body, and that the rectal temperature was ·6° to ·8° higher than that in the axilla. The figures given by Wunderlich[8] for the mean temperature of the rectum, mouth, and axilla are 37°·3, 37°·15, and 37° respectively. Redard,[9] on the other hand, states that the temperature of the mouth is ·2° higher than that in the axilla, and ·3° to ·6° lower than that of the rectum. Neuhauss[10] found, as the result of forty comparative experiments, in which the temperature was observed simultaneously in the rectum and in the axilla, that the rectal was 0°·6 higher than the axillary temperature.

We may take as our guide the averages obtained from the results of different observers, and conclude that the rectum has a temperature

[1] *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 558.
[2] *Proc. Roy. Soc. London*, 1869–70, vol. xviii. p. 368.
[3] Thèse de Paris, 1873, quoted from Richet, *Rev. scient.*, Paris, 1885, tome ix. p. 433.
[4] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xvi. S. 101.
[5] *Ibid.*, 1869, Bd. ii. S. 387.
[6] "Handbuch d. Path. u. Therap. des Fiebers," S. 44.
[7] "De la temperature du corps humain," Paris, 1877, tome i. p. 434.
[8] "Medical Thermometry."
[9] "Études de thermometrie clinique," 1874, p. 20.
[10] *Virchow's Archiv*, 1893, Bd. cxxxiv. S. 365.

0°·4 above that of the mouth, and that the difference between the temperature of the axilla and of the mouth is so small that it may be neglected, especially since the variation is not constantly in favour of the one or the other.

The temperature of internal parts in animals.—Numerous observations have been made upon the temperature of the internal parts of animals, either during life or immediately after death. Some of these results are now given in the following tables:[1]—

Animal.	Temperature of Part.	Observer.
Dog	Rectum, 38°	Hunter.[2]
	Right ventricle, 38°·3	,,
	Liver, 38°·2	,,
	Stomach, 38°·3	,,
Dog	Aorta, 38°·6	Bernard.
	Portal vein, 38°·8	,,
Lamb just dead	Rectum, 40°·8	Davy.[3]
	Liver, 41°·4	,,
	Lung, 41°·4	,,
	Right ventricle, 41°·1	,,
	Left ventricle, 41°·7	,,
	Blood of jugular vein, 40°·8	,,
	Blood of carotid artery, 41°·7	,,
Dogs	Blood in abdominal aorta, 38°·3–38°·6	Bayliss and Hill.[4]
Lambs (four) just dead	Rectum, 40°, 40°·6, 40°·6, 40°·6	Davy.[3]
	Right ventricle, 40°·8, 40°·6, 40°·8, 41°·1	,,
	Left ventricle, 41°·1, 41°·1, 41°·4, 41°·7	,,
	Blood of jugular vein, 40°	,,
	Blood of carotid artery, 40°·8	,,
Dog	Portal vein, 40°·2	Bernard.[5]
	Hepatic vein, 40°·6	,,
Lambs (three), just dead	Brain, 40°, 41°, 40°·8	Davy.[3]
	Rectum, 40°·4, 40°·8, 41°·4	,,
Turkey, just dead	Cloaca, 42°·2	Davy.[6]
	Gizzard, 42°·8	,,
	Pectoral muscle, 42°·2	,,

Hobday[7] finds in the case of horses, cows, sheep, dogs, and pigs, that the vaginal temperature is generally one-tenth of a degree lower than that of the rectum; at the times of œstrum, however, the vagina often has the higher temperature.

The temperature of arterial and venous blood.—The temperature of the blood has attracted considerable attention for many years—first, on account of the ancient view that the heat of the body was produced in the heart; and, secondly, because the work of Lavoisier and Crawford tended to show that heat was produced in the blood as it passed through the lungs or other parts of the body. More recently, attention has again been directed to this question by Berthelot,[8] who shows that a certain amount of heat is formed in the lungs by

[1] The results of other observations will be found in Rosenthal's article, Hermann's "Handbuch," Bd. iv. Th. 2, S. 393.

[2] "Works," Palmer's edition, London, 1837, vol. iv. p. 145.

[3] "Researches," London, 1839, vol. i. p. 147; *Phil. Trans.*, London, 1814, p. 590.

[4] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 351.

[5] "Leçons sur la chaleur animale," 1876, p. 188.

[6] "Researches," London, 1839, vol. i. p. 159.

[7] *Vet. Record*, London, 1896, vol. viii. p. 488.

[8] This article, p. 839.

the combination of oxygen with hæmoglobin. The numerous results obtained by different observers have been collected by Bernard,[1] and are given in the following tables:—

TABLE I.

Results in which the Arterial Blood is warmer than the Venous.

Author.	Arterial Blood.	Venous Blood.	Difference.	Animal.	Part Examined.	Method.
Haller[2] (1760), Schwenke	37°·2	36°·1	1°·1	?	?	?
Crawford[3] (1778)	38°·8	37°·5	1°·3	Sheep.	Carotid artery, jugular vein.	Thermometer placed in blood collected.
Krimer (1823)	38°·18	37°·20	0°·98	Man.	Temporal artery, jugular vein.	Thermometer in jet of blood.
	37°·5	36°·6	0°·9	Woman.	,,	,,
	37°·2	36°·3	0°·9	Man.	Amputation of arm, brachial artery and vein.	,,
Scudamore[4] (1826)	37°·7	36°·6	1°·1	Sheep.	Carotid artery, jugular vein.	,,
	36°·1	35°·5	0°·6	Man.	Temporal artery, vein of arm.	,,
Saissy[5] (1808)	38°·5	38°·0	0°·5	Marmot.	Right and left ventricle.	Incision of heart.
	36°·5	36°·0	0°·5	Hedgehog.	,,	,,
	38°·0	37°·5	0°·5	Squirrel.	,,	Experiments on two animals compared.
	31°·4	31°·0	0°·4	Bat.	,,	,,
J. Davy[6] (1815)	40°·0	39°·1	0°·9	Lamb.	Carotid artery, jugular vein.	One thermometer in vein, another in jet of arterial blood.
	40°·5	40°·0	0°·5	,,	,,	,,
	40°·5	40°·0	0°·5	,,	,,	,,
	40°·5	39°·7	0°·8	,,	,,	,,
	40°·5	40°·0	0°·5	,,	,,	,,
	40°·2	39°·7	0°·5	Ewe.	,,	,,
	40°·0	39°·1	0°·9	,,	,,	,,
	40°·0	39°·4	0°·6	,,	,,	,,
	38°·6	37°·7	0°·9	Ox.	,,	,,
	38°·3	38°·3	0°·0	,,	,,	,,
Nasse[7] (1843)	41°·1	40°·8	0°·3	Lamb.	Right and left ventricle; great intestine, 40°·0	Animals just dead, chest opened, ventricles incised.
	41°·1	40 ·5	0°·6	,,	40°·5	
	41°·1	40°·8	0°·3	,,	40°·5	
	42°·8	41°·25	1°·55	?	,,	,,
	41°·8	40°·6	1°·2	?	,,	,,
	42°·5	41°·25	1°·25	?	,,	,,

[*Continued on next page.*

[1] "Leçons sur la chaleur animale," 1876, p. 40 *et seq.*
[2] "Elementa Physiol.," 1760.
[3] "Experiments and Observations on Animal Heat," London, 1779.
[4] "An Essay on the Blood," London, 1824.
[5] "Recherches expérimentales," etc., Paris, 1808, p. 69.
[6] *Phil. Trans.*, London, 1814.
[7] *Rheinisch. u. Westphal. Correspondenzbl.*, 1843, 1844, 1845.

TABLE I.—*continued.*

Author.	Arterial Blood.	Venous Blood.	Difference.	Animal.	Part Examined.	Method.
Becquerel and Breschet[1] (1839)	...	...	0°·84	Dog.	Aorta where it left heart; inferior vena cava where it entered heart.	Thermo-electric needles, chest opened in animals just dead.
	...	...	1°·12	,,	Crural artery and vein.	,,
	...	...	0°·84	,,	,,	,,
	...	...	0°·84	,,	Carotid artery, crural vein.	,,
	38°·90	38°·0	0°·90	,,	Crural artery and jugular vein.	,,

TABLE II.

Results in which the Venous Blood is warmer than the Arterial.

Author.	Arterial Blood.	Venous Blood.	Difference.	Animal.	Part Examined.	Method.
Berger[2] (1833)	40°·90	41°·40	0°·50	Sheep.	Right and left ventricle.	Not stated.
Collard de Martigny,[3] and Malgaigne (1832)	...	...	1°·0	Dog.	,,	Animal just dead; chest partly open.
Magendie and Claude Bernard (1844)	...	...	...	Horse.	Right ventricle warmer than left.	Animal alive; circulation not interrupted.
Claude Bernard (1849)	...	...	...	Dog.	Inferior vena cava at level of liver warmer than aorta.	Animal alive; thermometer introduced by the abdomen.
Hering[4] (1850)	38°·77	39°·30	0°·53	Calf with ectopia of heart.	Right and left ventricle.	Incision of ventricles.
G. Liebig[5] (1854)	36°·32	36°·35	0°·3	Dog.	,,	Animal alive; circulation not interrupted; thermometers introduced by vessels of neck
Claude Bernard (1857)	38°·0	38°·2	0°·2	Dog.	,,	,,
	39°·3	39°·5	0°·2	,,	,,	,,
	39°·1	39°·2	0°·1	,,	,,	,,
	38°·6	38°·8	0°·2	,,	,,	,,
	38°·5	38°·7	0°·2	,,	,,	,,
	38°·6	38°·8	0°·2	,,	,,	,,
	39°·1	39°·2	0°·1	,,	,,	,,
	38°·7	38°·9	0°·2	,,	,,	,,
	38°·8	38°·9	0°·1	,,	,,	,,
	39°·2	39°·4	0°·2	,,	,,	,,
	40°·12	40°·37	0°·25	Sheep.	,,	,,
	39°·92	40°·32	0°·40	,,	,,	,,
	39°·58	39°·60	0°·02	,,	,,	,,
	40°·24	40°·39	0°·15	,,	,,	,,
	39°·58	39°·87	0°·29	,,	,,	,,
	40°·09	40°·48	0°·39	,,	,,	,,

[1] *Ann. d. sc. nat.*, Paris, "Zool.," Sér. 2, tomes iii. and iv.
[2] *Mém. Soc. de phys. et d'hist. nat. de Genève*, 1833, tome vi. p. 353.
[3] *Journ. compl. d. sc. méd.*, Paris, 1832, tome xliii. p. 386.
[4] *Arch. f. physiol. Heilk.*, Stuttgart, 1850.
[5] "Ueber die Temperaturunterschiede des venosen und arteriellen Blutes," Giessen, 1853.

It will be seen from the two tables that the results lead to directly opposed conclusions, but a critical examination shows that the correct one is probably that the blood in the right ventricle is 0°·1 to 0°·2 warmer than that in the left. In many of the older experiments the methods were inexact, the chest was opened and the heart exposed; the right ventricle, on account of its thin walls, would cool more quickly than the left, as shown experimentally by G. Liebig. The most exact method appears to be the insertion of delicate thermometers or thermo-electric needles down the jugular vein and the carotid artery into the right and left ventricle respectively. This method was employed by Heidenhain and Korner[1] in numerous experiments upon dogs, with the result that in all but one of the observations the right side of the heart was warmer than the left. Thus in one case the difference was ·6°, in two ·5° to ·6°, in three ·5°, in five ·3° to ·4°, in twenty-seven ·2° to ·3°, in thirty-six ·1° to ·2°, in twenty-one ·15°, in one case no difference at all. To determine whether the inspiration of cold air was the cause of this difference, Heidenhain and Korner made comparative experiments, employing for artificial respiration in the one case cold air (17°), and in the other hot air (40°) saturated with moisture. The difference still remained, and it was therefore concluded that respiration was not the cause; cold air when inspired is warmed and saturated with moisture before it reaches the alveoli;[2] further, in passing through the upper parts of the respiratory tract, the cold air would cool the blood in veins going to the superior vena cava and thus to the right side of the heart. These observers conclude that in the dog the right ventricle is warmer than the left, because its walls lie nearer to the liver and other abdominal organs, which have a high temperature, while the left ventricle is surrounded by lung. It was found, in fact, that the difference in temperature could be reduced to a minus quantity by artificially lowering the temperature of the abdominal cavity. Bernard does not accept this explanation as satisfactory; for he points out that in Hering's observation the right ventricle was half a degree warmer than the left, although the heart, owing to a congenital defect, was outside the thorax.

The temperature of the skin.—The temperature of the human skin shows differences in different parts of the body, and is also subject to variations due to alterations in the external temperature, the amount of natural or artificial covering, the vascularity of the parts, and the amount of evaporation taking place from the surface. Apart from these variations, there is a difficulty in measuring accurately the temperature of the skin; a mercurial thermometer applied to the skin receives heat from the surface in contact with the skin, and loses heat from the surface exposed to the air. If, on the other hand, the thermometer is covered with a non-conductor, or the external temperature is raised, then the heat of the part of the skin observed is increased. To overcome these difficulties, thermo-electric methods have been used.[3]

The disadvantages of these thermo-electric methods are the complexity

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1871, Bd. iv. S. 558.

[2] See "Chemistry of Respiration," this Text-book, vol. i. p. 754.

[3] Christiani and Kronecker, *Arch. f. Physiol.*, Leipzig, 1878, S. 334; Kunkel, *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 55; Masje, *Virchow's Archiv*, Bd. cvii. S. 17, 267; Geigel, *Verhandl. d. phys.-med. Gesellsch. in Würzburg*, 1888, N. F., Bd. xxii. S. 8; Stewart, *Stud. Physiol. Lab. Owens Coll.*, Manchester, 1891, vol. i. p. 100.

of the apparatus required, the necessity of graduation, and the time taken in observation. Bayliss and Hill[1] found that the wire-resistance thermometer[2] could not be employed for the investigation of changes of temperature in a warm-blooded animal; the slightest movements, as those of artificial respiration, in the curarised animal producing deflections of the galvanometer. A flat mercurial thermometer, on the other hand, is easily applied, and furnishes comparative data of considerable value.[3]

Some of the earliest experiments with mercurial thermometers were made by J. Davy, who obtained the following results, when the temperature of the room was 21° :—

Sole of the foot	32°·2	Middle of the rectus femoris	32°·78
Between internal malleolus and tendo Achillis	33°·89	Groin	35°·84
Middle of tibia	33°·06	One inch below navel	35°·00
Middle of calf	33°·89	Left sixth rib over heart	34°·44
Bend of the knee	35°·00	Right sixth rib	33°·89
Middle of the thigh	34°·44	Axilla (closed)	36°·67

Kunkel[4] used a thermo-electric method, which was exact to about 0°·1, and obtained the following results for the temperature of different parts of the skin of a healthy muscular man, 35 years of age, 179 cm. in height, and 84 kilos. in weight. The temperature of the room was 20° :—

Forehead	34°·1–34°·4	Arm	34°·3
Over malar bone	34°·1	Sternum	34°·4
Cheek under malar bone	34°·4	Over pectoralis major	34°·7
Lobe of ear	28°·8	Over heart	34°·6
Back of hand	32°·5–33°·2	Right iliac fossa	34°·4
Palm of hand (closed for some time)	34°·8–35°·1	Left „	34°·6
Palm of hand (open)	34°·4–34°·8	Back, over sacrum	34°·2
Wrist	33°·1	„ over ribs	34°·5
Forearm	33°·7	Buttock	32°·0[5]
„ upper part	34°·0	Thigh	34°·2
		Calf	33°·6

Experiments were also made upon the effect of exposure to cold. Thus, after the man lightly clothed had taken a walk for half an hour in a cold, sharp, north-east wind (–5°), the following temperatures were observed—face, 27°·7–28°·7; back of hand, 24°·7; chest and abdomen, 32°·1; arm, 30°·7–31°·1; but after he had remained for forty minutes in a room at 15°, the face had a temperature of 34°·6, the back of the hand 31°·2, and the abdomen 33°·9.

Working the muscles of one arm raised the temperature of the skin

[1] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 352.

[2] Rolleston, *ibid.*, 1890, vol. xi. p. 208.

[3] Davy, *Phil. Trans.*, London, 1814, vol. civ. p. 590; "Researches," London, 1839, vol. i. p. 150; Alvarenga, "Précis de thermométrie clinique générale," 1871, p. 45; Waller, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894, vol. xv.; Hale White, Croonian Lectures, *Lancet*, London, June 19th, 1897, *Brit. Med. Journ.*, London, 1897, vol. i. p. 1654; Pembrey, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi.

[4] *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 55.

[5] This low reading Kunkel attributes to the loss of heat by conduction when the man was sitting down.

of that part above 34°, whereas the temperature of the abdomen was only 32°·5. The highest temperature observed in healthy men was 35°·6, on the skin of the face.

Kunkel concludes from his observations that the temperature of the human skin is almost constant, and that the temperature of the body is regulated to a very slight degree by changes in the temperature of the skin.

The Regulation of Temperature.

Inasmuch as the constancy of temperature varies in different animals, and even in the same animal under different conditions, such as age and hibernation, so also various grades of perfection are observed in the power of regulation. In man this power is so greatly developed that his temperature is almost the same, whether he lives in the Arctic regions, with an external temperature 50° below zero, or in the Tropics, where the temperature of the air may be as high as 48°. For shorter periods a man can remain in a room heated to 121° without the temperature of his body rising above the normal.[1] Other mammals have a less perfect regulation, as shown by the greater variations of their temperature.

In young immature mammals and birds the power of regulation is imperfect, for when they are exposed to cold their temperature falls, and they pass into a condition in which they resemble the cold-blooded animals, their temperature rising and falling with that of their surroundings. A similar imperfection in regulation is seen in some mammals during hibernation. Lastly, in the so-called cold-blooded animals, there are various grades in this capacity for regulating temperature, as is shown by the high temperature of bees in winter, when compared with that of most of the lower animals, in which there is a mere trace of regulation.

Even in those warm-blooded animals which possess a perfect power of heat regulation, there are limits to this power. If the animal be exposed to excessive cold, the loss of heat is great, and only within certain limits can compensation be effected by an increased production of heat. When compensation fails, then the animal's temperature falls, its bodily and mental activities are diminished, and it passes into a sleepy, unconscious condition which ends in death. Such a condition is observed in men or animals before they are "frozen to death."

On the other hand, extreme heat can only be resisted within a certain range; the production of heat in the body can be diminished, but not suspended; the loss of heat can be greatly increased by sweating and by a greater exposure of blood in the vessels of the skin, but if the air be of a temperature equal to, or nearly equal to, that of the body, and greatly laden with moisture, then the loss of heat is slight or even suspended. Under such circumstances the internal temperature of the animal rises rapidly to a point incompatible with life. The extremes of heat and cold which can be borne without injury to life, have already been discussed.

The mean temperature of the higher animals is fairly constant under very great differences of external temperature, and to maintain such a condition the loss and the production of heat must be almost equal. That there is no perfect equality has already been shown in the daily

[1] Bladgen, *Phil. Trans.*, London, 1775, vol. lxv. p. 484. This article, p. 814.

variation of the temperature of the body, in the rise of temperature observed after exercise, and during residence in tropical climates.

The regulation of temperature, therefore, embraces two processes—regulation by varying loss of heat, regulation by varying production of heat.

The regulation of heat production.—In considering the regulation of heat production, it is necessary to trace out briefly the various discoveries which have established, as a fact, that animal heat is due to combustion within the tissues.

Historical account of facts and theories upon the sources of animal heat.[1]—The ancients considered animal heat to be beyond the reach of physical and chemical laws. They could assign no cause for it, and therefore looked upon it as some innate quality, something essentially "vital." This "vital" heat was supposed to be concentrated in the heart (Plato, Aristotle, Galen), and to be distributed to the body by the blood in the veins. It was prevented from accumulating by respiration, the chief function of which was to cool and temper the blood.

As knowledge in physical and chemical processes increased, attempts were made to give a rational explanation of animal heat. It was well known that heat arose during fermentation, and by the contact of acid and base; animal heat was therefore considered to arise by some similar process or processes taking place in the blood. Willis,[2] about the year 1670, put forward the theory that there is in the blood a combustion which depends upon the fermentation excited by the combination of different chemical substances. Friction was another well-known source of heat, and was the explanation given by Boerhaave;[3] he considered that animal heat was due to the friction of the blood corpuscles in the vessels. Stephen Hales[4] adopted this theory, and gave certain experiments, which he thought supported it.

A much more correct opinion had already been formed in 1674 by Mayow,[5] who, after his experiments on the constitution of air and its relation to the heat of combustion, extended the analogy of combustion to animal heat. He held that the function of the lungs was not to cool the blood, but to enable that fluid to absorb the nitro-aerial gas (oxygen) of the air, and so generate heat.

Later research has shown that the heat of living things is not due to any mystical so-called "vital" force, but to the processes of combustion, which form one of the most important phenomena of life. The different steps by which this knowledge has been attained are found in the discovery of Black,[6] that carbon dioxide was produced in animals by a process of combustion; in the work of Lavoisier[7] and Crawford,[8] who showed that the heat of an animal might be accounted for by the processes of combustion; in the researches of Dulong[9] and Despretz,[10] whose results, when critically examined and explained by Liebig,[11] formed an important support for the law of the conservation of energy.

[1] Accounts of the old theories will be found in C. Bostock, "Essay on Respiration"; "An Elementary System of Physiology," 2nd edition, 1828, vol. ii. p. 243; Gavarret, "De la chaleur produite par les êtres vivants," Paris, 1855; and "Les phenomènes physiques de la vie," Paris, 1869; Lorain, "De la temperature du corps humain," Paris, 1877, vol. i. p. 39; Rubner, *Ztschr. f. Biol.*, München, 1893–94, Bd. xxx. S. 73.

[2] "De Accensione Sanguinis." [3] "Aphor. cum Notis Sweiten," pp. 382, 675.

[4] "Statical Essays," 2nd edition, 1733, vol. ii. p. 90.

[5] "Tractatus Quinque," Oxonii, 1674.

[6] "Lectures on Chemistry," edited by Robison, Edinburgh, 1803.

[7] *Hist. Acad. roy. d. sc.*, Paris, 1777.

[8] "De calore Animali," 1779; "Experiments and Observations on Animal Heat," 1788.

[9] *Ann. de chim. et phys.*, Paris, 1843, Sér. 3, tome i. p. 440.

[10] *Ibid.*, 1824, Sér. 2, tome xxvi. p. 337. [11] "Thierchemie," S. 28.

Helmholtz, Ludwig, Pflüger, and others, by their investigations upon the production of heat in muscle, glands, and other tissues, and their determinations of the respiratory exchange of animals, have indicated where and how heat is produced. Finally, the exact determinations made by Rubner[1] upon heat production and metabolism have proved that chemical change is the cause of animal heat. Simultaneous determinations of the exchange of material and the production of heat in dogs, under different conditions as regards diet, were made, and the results show that the heat of combustion of the food, as determined in a calorimeter, is equal to the heat given off by the animal; in fact, the animal must be looked upon as a living calorimeter, in which the food is burnt. The results are so exact that they prove the conservation of energy in a vital process.

Condition of the Animal.	Heat as Calculated.	Heat as found by Calorimeter.	Percentage Difference.
Fasting . . .	1296·3 cal.	1305·2	+0·69
Diet of fat	1510·1 ,,	1495·3	−0·97
Diet of flesh and fat . .	2492·4 ,,	2488·0	−0·17
Diet of flesh . . .	4780·8 ,,	4769·3	−0·24

The above figures only give some of the results, but the mean of all the experiments shows that the amount of heat, as determined directly by the animal calorimeter, is only 0·47 per cent. less than the amount as calculated from the heats of combustion of the different substances which have been decomposed in the animal's body.

The Relation of Chemical Change to Heat Production.

A consideration of the law of the conservation of energy leads to the conclusion that the sole cause of animal heat is a chemical process, a combustion of food substances by the oxygen taken in by the animal; as just mentioned, the experimental proof of this conclusion has been recently given by Rubner. The chemical energy of the ingesta manifests itself chiefly in two forms, heat and motion.

In this connection it is important to consider the heats of combustion of the various substances which form part of an animal's body or food, for it will thereby be possible to determine indirectly the amount of heat produced by an animal. A given amount of chemical action is accompanied by the production or the absorption of a definite quantity of heat. The accurate determination of this quantitative relation is beset with considerable difficulties, for the chemical changes in the complex substances of animal tissues or food are rarely simple, and are accompanied by physical changes, which have to be measured and taken into account before the amount of heat due to the chemical change can be estimated. Chemical decomposition is attended with the absorption of a quantity of heat equal to that which would be evolved by the combination of the same chemical substances.[2] Therefore, in the

[1] *Ztschr. f. Biol.*, München, 1894, Bd. xxx. S. 135.

[2] Favre and Silbermann, *Ann. de chim. et phys.*, Paris, 1842, Sér. 3, tome xxxiv. p. 357; Woods, *London, Edinburgh, and Dublin Phil. Mag.*, London, 1851, vol. ii. p. 268, 1852, vol. iv. p. 370; Joule, *ibid.*, 1852, vol. iii. p. 481.

estimation of the production of heat during a complex chemical change, involving combination and decomposition, it is only necessary to consider the first and final conditions of the substances, whatever may have been the intermediate stages.[1]

The determination of the heat produced or absorbed by chemical change is made by enclosing the acting substances in a chamber surrounded by water or mercury, the rise or fall of temperature in which indicates the amount of heat produced or absorbed, as the case may be.[2]

The heat of combustion of substances of physiological interest has been determined by various observers;[3] the following table gives the values of some of the most important substances:—

Substance, 1 grm. (dry).	Heat of Combustion.	Authority.	Substance, 1 grm. (dry).	Heat of Combustion.	Authority.
Hydrogen .	33,881 cal.[4]	Andrews.	Casein . . .	5,855 cal.	Danilewsky.
,, .	34,662 ,,	Favre and Silbermann.	,, . . .	5,867 ,,	Stohmann.
			,, . . .	5,849 ,,	,,
Carbon . .	7,900 ,,	Andrews.	Cows' milk .	5,733 ,,	Danilewsky.
,, Wood-charcoal .	8,080 ,,	Favre and Silbermann.	Women's milk	4,837 ,,	,,
			Fat . . .	9,686 ,,	,,
Cheese . . .	6,114 ,,	Frankland.	,, . . .	9,423 ,,	Rubner.
Potatoes . .	3,752 ,,	,,	Dextrose . .	3,939 ,,	Rechenberg.[9]
,, . .	4,234 ,,	Danilewsky.[5]	Maltose . .	4,163 ,,	,,
Lean beef . .	5,313 ,,	Frankland.	Milk sugar .	4,162 ,,	,,
,, . .	5,724 ,,	Danilewsky.	Starch . . .	4,479 ,,	,,
,, . .	5,641 ,,	Stohmann.[6]	,, . . .	4,182 ,,	Stohmann.
,, . .	5,656 ,,	Rubner.[7]	Cane-sugar .	4,176 ,,	Danilewsky.
White of egg	4,896 ,,	Frankland.	Glycogen . .	4,190 ,,	Stohmann.
Yolk of egg .	6,460 ,,	,,	Cellulose . .	4,185 ,,	,,
Butter . .	7,264 ,,	,,	Urea . . .	2,537 ,,	Danilewsky.
Bread crumb.	3,984 ,,	,,	,, . . .	2,537 ,,	Stohmann.
Blood fibrin .	5,772 ,,	Danilewsky.	,, . . .	2,525 ,,	Berthelot and Petit.[10]
,, .	5,637 ,,	Stohmann.[8]			
Peptone . .	4,876 ,,	Danilewsky.	,, . . .	2,523 ,,	Rubner.[7]
,, . .	5,298 ,,	Stohmann.	Uric acid . .	2,741 ,,	Stohmann.
Serum albumin	5,917 ,,	,,	Hippuric acid	5,678 ,,	,,
Hæmoglobin .	5,885 ,,	,,	Fæces . . .	4,479 ,,	Rechenberg.

The above table shows that the different foodstuffs have different values as producers of heat, and from these it is possible to calculate the physical value of one kind of food in terms of the others. The

[1] Hess, quoted from Rubner (*Ztschr. f. Biol.*, München, 1894, Bd. xxx. S. 135).

[2] For further details on such calorimeters, see Miller, "Chemical Physics," p. 338, and Watts' "Dictionary of Chemistry," vol. iii. pp. 28, 103; Stohmann, *Journ. f. prakt. Chem.*, Leipzig (2), Bd. xix. S. 115; Bd. xxxix. S. 503.

[3] Crawford, "On Animal Heat," 1788, 2nd edition, pp. 320, 333, 351; Favre and Silbermann, *Ann. de chim. et phys.*, Paris, 1842, tome xxxiv. p. 357; Frankland, *London, Edinburgh, and Dublin Phil. Mag.*, London, 1866, vol. xxxii. p. 182; Hermann, *Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1868, S. 18, 84; Rubner, *Ztschr. f. Biol.*, München, 1885, Bd. xxi. S. 357; Berthelot, *Compt. rend. Acad. d. sc.*, Paris, 1886, tome cii. pp. 1211, 1284.

[4] Calorie = the heat required to raise 1 grm. of water 1° C.; kilo-calorie = 1000 calories = heat required to raise 1 kilo of water 1° C.

[5] *Arch. f. d. ges. Physiol.*, Bonn, 1885, Bd. xxxvi. S. 230.

[6] *Journ. f. prakt. Chem.*, Leipzig, Bd. xliv. S. 336.

[7] *Ztschr. f. Biol.*, München, 1893–94, Bd. xxx. S. 88.

[8] *Ibid.*, 1895, Bd. xxxi. S. 364.

[9] "Ueber die Verbrennungswärme organischer Substanzen," Leipzig, 1880.

[10] *Compt. rend. Acad. d. sc.*, Paris, 1889, tome cix. p. 759.

following table of isodynamic foodstuffs is taken from Danilewsky's work:—

	Fat.	Starch.	Grape-Sugar.	Cane-Sugar.	Cellulose.	Peptone.	Extract of Meat.
100 grms. casein =	61	133	151	142	133	121	135
100 grms. fat =	100	220	250	236	221	201	224
100 grms. starch =	46	100	114	107	100	92	102

The above data are for physical values. It is necessary, therefore, to determine how far the different foodstuffs undergo combustion in the living body, and what values they have as producers of heat during that combustion.

Rubner has shown that some of the products of the combustion of proteid escape in the fæces as well as in the urine; the heat value of these substances must be determined and deducted from the heat of combustion of proteid. The reduced or physiological heat value of 1 grm. of dry proteid is therefore only about 4000 calories. The fats and carbohydrates appear to undergo complete oxidation in the body.

An important series of experiments on the sources of animal heat has been performed by Rubner.[1] The experiments were carried on for several days in succession upon a dog weighing 12 kilos. The animal was given a known amount of meat once a day; the urine and fæces were collected and their heat of combustion determined, and the heat given off by the animal was measured by a calorimeter. At the same time the discharge of carbon dioxide and water from the dog were determined, also the total nitrogen lost in the urine and fæces, and the loss or gain in weight of the animal. No external work was done by the dog, for it remained quiet in the calorimeter, and therefore no energy was lost in the form of work.

The following is an example of the results obtained:—

Date.	Condition.	Total Discharge of Nitrogen.	Carbon from Fat.	Heat Calculated from Proteid.	Heat Calculated from Fat.	Total Heat in Twenty-four Hours.
16th October 1889	Fasting	3·06	16·38	77·0	201·5	278·5 kilo-cal.

This result, 278·5 kilo-calories, compares well with the heat, 276·8 kilo-calories, given off by the animal in the calorimeter.

Date.	Condition.	Heat given to Calorimeter.	Heat Lost in Ventilation.	Heat Lost in Evaporation of Water.	Total Heat in Twenty-four Hours.
16th October 1889	Fasting	213·2	17·6	45·9	276·8 kilo-cal.

[1] *Ztschr. f. Biol.*, München, 1893–94, Bd. xxx. S. 73.

Thus it is possible to calculate the production of heat in an animal, if the quantity and nature of its food and the amount of the discharge of nitrogen in the urine and fæces be known. This Rubner has done, and has compared the result with the heat given off by the animal to a calorimeter. Thus :—

Food of dog during 12 days = { 228·06 grms. proteid.
340·4 grms. fat.

In the urine 30·0 grms. N were discharged, and the dry fæces amounted to 16·8 grms.

Calculation 1, *from physiological heat values.*

Proteid, 228·06 × 4·0 kilo-cal. = 912·24
Fat, 340·4 × 9·423 „ = 3207·0

4119·2 kilo-cal. in 12 days.

Calculation 2, *from physical heat value with reduction for heat value of urine and fæces.*

Proteid . . = 1222 kilo-cal.
Fat . . . = 3207

4429
305·2 { 223·5 heat value of urine.
81·7 „ „ fæces.

In 12 days 4124 kilo-calories.

The amount of heat actually given off by the dog during this time was 3958 kilo-calories. Thus the calorimeter showed that 96 per cent. of the energy of the food had appeared as heat.

Recent work by Rubner[1] has shown that the body of a living animal may be looked upon as a calorimeter, and may be used as such for the determination of the heat of combustion of food. Thus the heat of combustion of 1 grm. of dry meat, determined in this way, is 4007 calories, that of 1 grm. of dry fat 9353 calories, figures which are practically the same as 4000 and 9423 respectively, the results obtained by combustion in a Thompson's calorimeter, when allowance is made for the heat value of the products of the proteid lost in the urine and fæces.

The following is one of Rubner's examples of such a determination :—A small dog fed upon meat discharged daily 10·09 grms. of nitrogen in its urine and fæces, and 9·06 grms. carbon from fat underwent combustion. The heat produced, as determined by the calorimeter, was 379·5 kilo-calories. On a diet of meat and fat the same dog discharged 2·95 grms. of nitrogen, and 19·12 grms. carbon from fat underwent combustion, while the production of heat was 311 kilo-calories. Now, if the calorimetric value of the nitrogen be represented by x and that of carbon from fat by y, then—

$$(1)\quad 10{\cdot}09x + 9{\cdot}06y = 379{\cdot}5$$
$$(2)\quad 2{\cdot}95x + 19{\cdot}12y = 311{\cdot}0$$

$\therefore\ x = 26{\cdot}7$ kilo-calories and $y = 12{\cdot}15$ kilo-calories.

The results obtained by direct combustion were 26·0 and 12·3 kilo-calories.

The heat corresponding to 1 grm. nitrogen = 6·493 grms. dry meat = 26·36 kilo-calories ; that to 1 grm. carbon from fat = 1·3 grm. fat = 12·16 kilo-calories.

[1] *Ztschr. f. Biol.*, München, 1894, Bd. xxx. S. 140.

The heat of combustion of food may be determined in three ways—(1) by direct estimation with a calorimeter, (2) by calculation from the oxygen necessary for oxidation, and (3) by measurement of the heat produced by the combustion of the food inside the animal body. Such determinations have been made by Rubner,[1] and the following table is the isodynamic value of 100 grms. of fat estimated by these three modes:—

	First Method.	Second Method.	Third Method.
100 grms. of Fat =			
Proteid . .	201	193	211
Starch . .	221	240	232
Cane-sugar .	231	249	234
Grape-sugar .	243	263	256

It is to be noted that, with the exception of proteid, all food substances give too low a value for the heat of combustion when it is calculated from the equivalents of oxygen necessary for combustion. The calculation of the heat of combustion from the oxygen necessary for oxidation gives results which are not exact.

The value of these calculations in the estimation of the heat produced in a living body will be seen by comparing the results with those obtained by direct determination with the calorimeter. The following are Vierordt's[2] calculations for the heat production of an adult man in twenty-four hours:—

(*a*) Calculation, according to Dulong's principle, from the heat of combustion of carbon and hydrogen.

An adult man consumes in twenty-four hours—

	C.	H.	N.	O.
120 grms. proteid . . .	64·18	8·60	18·88	28·34
90 ,, fat . . .	70·20	10·26	...	9·54
330 ,, carbohydrate .	146·82	(Hydrogen	combined wi	th oxygen)
	281·20	18·86	18·88	...
In urine and fæces . .	29·80	6·3	...	...
	251·4	12·56	...	...

251·4 grms. C. × 8,080 . . = 2,031,312 calories.
12·56 ,, H. × 34,460 . . = 432,818 ,,

Total heat production . . = 2,464,130 ,,

[1] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 386.
[2] "Grundriss der Physiol.," S. 281.

(*b*) Calculation, according to Frankland's principle, from the heat of combustion of food substances—

120 grms. proteid . . .	=	599,760 calories.
90 „ fat	=	816,210 „
330 „ carbohydrate . .	=	1,081,410 „
		2,497,380
41 grms. urea	=	83,066
Total heat production . .	=	2,414,314 calories.

In the consideration of the calculations by Vierordt it is necessary to remember that Dulong's principle only leads to approximate results, and that the values for the heat of combustion employed in the calculation according to Frankland's principle have been superseded by more recent and exact determinations. For this reason the following calculation is given:—

120 grms. proteid	× 4000 . . .	=	480,000
90 „ fat	× 9423 . . .	=	848,070
330 „ carbohydrate	× 4182 . . .	=	1,380,060
Heat produced by an adult man in twenty-four hours		=	2,708,130 calories.

The calculations of other observers give the following values:—

Calories for an adult man in 24 hours . . .	2,732,000	...	Helmholtz.[1]
	2,706,076	...	Ludwig.[2]
	1,800,000	Minimum of nourishment	Danilewsky.[3]
	3,210,000	Mixed diet— Ordinary work	,,
	3,646,007	Liberal diet— Hard work	,,
	3,780,000	Liberal diet— Very hard work	,,
	2,843,000	...	Rubner.

Scharling, from direct calorimetric observation, found that an adult man at rest gave 132,000 calories in an hour, 3,168,000 in twenty-four hours; and Hirn obtained the following results, 140,000 to 170,000 calories per hour, 3,360,000 to 4,080,000 calories in twenty-four hours.

The Specific Heat of the Body.

The first determinations of the specific heat of animal and vegetable tissues appear to have been made by Crawford.[4] The

[1] Encyclop. Wörterb. d. med. Wissensch., 1846, Bd. xxxv. S. 523.
[2] "Lehrbuch der Physiol.," S. 747.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1883, Bd. xxx. S. 175.
[4] "On Animal Heat," 1788, 2nd edition, p. 139. Determinations were also made by Kirwan and Dalton.

following are some of his results, and also those obtained recently by Rosenthal:[1]—

Crawford.		Rosenthal.	
Lean beef . . .	= 0·740	Compact bone . .	= 0·300
Hide of an ox with the hair	= 0·787	Spongy bone . .	= 0·710
Lungs of a sheep . .	= 0·769	Fat	= 0·712
Fresh milk of a cow .	= 0·999	Voluntary muscle .	= 0·825
Arterial blood of a dog .	= 1·030	Defibrinated blood .	= 0·927
Venous blood . .	= 0·8928		

It is to be noted that Davy,[2] Hillersohn, and Stein Bernstein[3] were unable to find any marked difference between the specific heats of arterial and venous blood. Recently Hale White[4] has made an ingenious attempt to obtain the specific heat of a living warm-blooded animal by experimenting upon a hibernating dormouse. His results vary between 0·812 and 1·18, but they are only approximately accurate, for the dormouse, even during hibernation, produces a small amount of heat.

Since all the tissues of the body contain a quantity of water, the mean specific heat must be near unity, probably about 0·83.

The Seats of Heat Production.

The work of Mayow (1674), Black (1757), Priestley (1772), Lavoisier (1777), and Crawford (1779)[5] led to the conclusion that animal heat was due to a process of combustion occurring in the body, but concerning the chief seat of this combustion there was no unanimous opinion. Mayow considered that the oxidation took place in the tissues all over the body; Crawford held that the heat was set free chiefly in the capillaries of the body, owing, as he thought, to a difference in the specific heat of arterial and venous blood; Lavoisier was at first undecided, and considered that the heat arose in the lungs, and possibly in other parts of the body, but finally he maintained that the lungs were the chief seat of combustion. The theory of Lavoisier was contested by Lagrange,[6] who maintained that if all the heat of the body were produced in the lungs, the tissues of that organ would be destroyed by so high a temperature. This objection was for long held to be fatal to Lavoisier's theory, until Berthelot,[7] by a careful calculation, showed that, granting all the heat to be formed in the lungs, the temperature of those parts would not be raised more than a minute fraction of a degree, owing to the great volume of air and blood in the lungs and the rapidity of the circulation, whereby the heat would be quickly distributed. Moreover, Berthelot has shown by experiment that a small amount of heat is formed in the lungs by the combination of oxygen with

[1] *Arch. f. Physiol.*, Leipzig, 1878, S. 215.
[2] "Researches," London, 1839.
[3] *Arch. f. Physiol.*, Leipzig, 1896, S. 249.
[4] *Journ. Physiol.*, Cambridge and London, 1892, vol. xiii. p. 789; Croonian Lectures, *Lancet*, London, June 19, 1897; *Brit. Med. Journ.*, London, 1897, vol. i. p. 1653.
[5] Mayow, "Tractatus Quinque," 1674; Black, "Lectures on Chemistry," edited by Robison, Edinburgh, 1803; Priestley, *Phil. Trans.*, London, 1772, vol. lxii. p. 147; Crawford, "De Calore Animali," 1779; "On Animal Heat," 2nd edition, 1788; Lavoisier, *Brit. Acad. roy. d. sc.*, Paris, 1777.
[6] Hassenfratz, *Ann. de chim.*, Paris, 1791, tome ix. p. 275.
[7] *Compt. rend. Acad. d. sc.*, Paris, 1889, tome cix. p. 776.

hæmoglobin.[1] He found in two experiments that 100 volumes of blood absorbed respectively 20·2 and 18·5 volumes of oxygen, and produced thereby 14·63 and 14·91 calories. Now, the combustion of the oxygen with carbon would produce 97·65 calories, but, in the formation of oxyhæmoglobin, only 14·8 calories were set free—that is, only a seventh of the heat of combustion would be set free in the lungs, the remaining six-sevenths in the tissues. M'Kendrick[2] and Bottomley have also been able, with a thermo-electric arrangement, to detect the heat produced by the union of hæmoglobin with oxygen.

The production of heat in muscle.—It has already been shown that during active muscular work the temperature of the body is slightly raised, although the loss of heat is at the same time greatly increased. The muscles must therefore be an important source of heat, and a further consideration will show that they are the chief source. The bulk of the body is chiefly composed of muscle; thus, in a dog weighing 11,700 grms., the muscles weigh 5400 grms., and the bones 2400 grms. (Bernard);[3] and even in a much less compact animal, a bat weighing 19·94 grms., the muscles weigh 6·378 grms. (Pembrey).[4]

The production of heat as one of the phenomena of contraction in a single isolated muscle, and the relation of heat to work during a single contraction and during tetanus, are considered elsewhere. Here the muscles have to be examined as seats of heat production, not only during contraction, but during apparent rest; and, further, as regards the part they play in the production and regulation of the warmth of the body.

The muscles, even when they have been removed from the body, are the seat of an energetic combustion (Humboldt,[5] Liebig,[6] Du Bois Reymond, Valentin,[7] Matteucci[8]). The following comparative experiments were made by Paul Bert.[9] Different tissues were removed from a dog just killed, and the absorption of oxygen and discharge of carbon dioxide were determined during a period of twenty-four hours, at a temperature varying from 0° to 10°:—

100 grms.	of muscle	absorbed	50·8	c.c. of oxygen,	and discharged	56·8	c.c. of carbonic acid.
,,	brain	,,	45·8	,,	,,	42·8	,,
,,	kidney	,,	37·0	,,	,,	15·6	,,
,,	spleen	,,	27·3	,,	,,	15·4	,,
,,	testis	,,	18·3	,,	,,	27·5	,,
,,	broken bone and marrow	,,	17·2	,,	,,	8·1	,,

Regnard[10] has shown that the respiratory exchange of isolated muscle rises and falls with the external temperature; at 10° the discharge of carbon dioxide by 1 kilo. of muscle is 40 c.c. in one hour, at 25° it is 129 c.c., and at 35° it amounts to 294 c.c., but above 40° the discharge decreases.

[1] See also Davy, "Researches," London, 1839, vol. ii. p. 168.
[2] *Brit. Med. Journ.*, London, 1888, vol. ii. p. 338.
[3] "Leçons sur la chaleur animale," 1876, p. 140.
[4] *Journ. Physiol.*, Cambridge and London, 1895–96, vol. xix. p. 485.
[5] "Versuche ueber die gereizte Muskel-und Nervenfaser," Berlin, 1797.
[6] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1850, S. 393.
[7] *Arch. f. physiol. Heilk.*, Stuttgart, 1855, Bd. xiv. S. 431.
[8] *Compt. rend. Acad. d. sc.*, Paris, 1856, tome xlii. p. 648; *Ann. de chim. et phys.*, Paris, 1856, tome xlvii. p. 129.
[9] "Leçons sur la physiol. comparée de la respiration," 1870, p. 46.
[10] "Recherches expérimentales sur les combustions respiratoires," Paris, 1879, p. 23.

Similar results have been obtained by Rubner[1] in limbs through which an artificial circulation of blood was maintained.

Although, as Hermann[2] has shown, some of the carbon dioxide may arise from the action of bacteria, yet these experiments show that even excised muscle is the seat of an energetic metabolism and of heat production. Tissot[3] has proved that the absorption of oxygen and the discharge of carbon dioxide occur in an excised muscle, even when every precaution is taken to maintain asepsis. The results of Minot's[4] experiments upon the production of carbon dioxide in resting and active muscle are opposed to those obtained by Paul Bert and Regnard, but his method has been shown by Zuntz[5] to be open to serious objections.

The subject of respiration in muscle will be discussed more fully in other parts of this work,[6] here it is only necessary to point out that the respiratory exchange of a muscle, even during apparent rest, is very marked, and becomes enormously increased during activity. This is well shown by the experiments of Sczelkow,[7] von Frey,[8] Chauveau and Kaufmann,[9] Hill and Nabarro.[10]

		Difference between Venous and Arterial Blood.		
		Muscle at Rest.	Muscle Active.	
Sczelkow	CO_2	+6·71	+32·37	
	O_2	−9·00	−36·78	
Chauveau and Kaufmann	CO_2	+8·70	+30·60	
	O_2	−11·40	−40·95	
			Tonic.	Clonic.
Hill and Nabarro	CO_2	+8·76	+41·70	+57·99
	O_2	−12·92	−41·25	−37·89

The muscles during apparent rest are in a state of tone, and are the seat of an energetic combustion, and therefore of heat production.

Further evidence of the important part played by the muscles in the production of heat is found in the fact that any cause which suspends the activity of the muscles, or more correctly the neuro-muscular system, lowers the temperature of the body. Curari causes muscular paralysis and a fall in the temperature of the body;[11] the respiratory exchange is greatly diminished, even if the animal's temperature is

[1] *Arch. f. Physiol.*, Leipzig, 1885, S. 38; this Text-book, article "Chemistry of Respiration."

[2] "Untersuch. u. d. Stoffwechsel der Muskeln," Berlin, 1867, S. 37.

[3] *Arch. de physiol. norm. et path.*, Paris, 1894, tome xxvi. p. 838; 1895, tome xxvii.

[4] "Die Bildung der CO_2 innerhalb des ruhenden und erregten Muskeln."

[5] Hermann's "Handbuch," Bd. iv. Th. 2, S. 96.

[6] See articles on "Chemistry of Respiration" and on "Metabolism," this Text-book, vol. i.

[7] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlv.

[8] *Arch. f. Physiol.*, Leipzig, 1885, S. 533.

[9] *Compt. rend. Acad. d. sc.*, Paris, 1886, tome ciii. pp. 974, 1057, 1153.

[10] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 218.

[11] Tscheschichin, *Arch. f. Anat. Physiol. u. wissensch. Med.*, 1866, S. 159. During the convulsions which are at first caused by curari the temperature rises; Bernard, "Leçons sur la chaleur animale," 1876, p. 157; Velten, *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxi. S. 361.

artificially maintained at the normal height.[1] These experiments have been extended by Pflüger,[2] who found in curarised rabbits that the intake of oxygen and the output of carbon dioxide fell respectively to 35·2 and 37·4 per cent. of the normal exchange: a rise in the temperature of the surroundings caused an increase in the respiratory exchange, and in the temperature of the animal, whereas a fall in the external temperature produced the opposite effects. These phenomena were not due to diminished supply of oxygen, for artificial respiration was maintained, the heart beat strongly, and the venous blood was brighter than in the normal animal. Further, it is not due to poisoning of the muscle substance itself, for Colasanti[3] found that the oxidation in the muscles of a limb with an artificial circulation was the same whether the blood did or did not contain curari. Similar results have been obtained with anæsthetics and drugs which depress the activity of the nervo-muscular system.[4]

It will be shown later that section of the spinal cord or of the motor nerves reduces a warm-blooded animal to a cold-blooded condition; its temperature falls, and it can no longer regulate its temperature. The completeness of the effect seems to depend upon the number of the muscles paralysed. On the other hand, calorimetric determinations show that muscular activity greatly increases the production of heat and the respiratory exchange.

It has already been stated that young mammals and birds, in which muscular co-ordination is well developed, are able to maintain their temperature at birth, whereas others born in a helpless condition resemble cold-blooded animals.

According to D. Macalister,[5] the muscles are fatigued as producers of heat sooner than as producers of work, and the effect of cold upon the muscles of anæsthetised mammals is to markedly depress the thermogenic function.

The involuntary muscular contraction in shivering causes a rise of temperature,[6] and this is especially noticeable in small thin dogs with little fur; in fact, shivering must be looked upon as an involuntary protective mechanism against cold.[7] In man, as Löwy[8] has shown, it may increase the metabolism by 100 per cent. The warming effect of muscular exertion is a matter of ordinary daily experience, and is well shown by the difference in the walk of a man during cold and hot weather.

The heat produced by the contraction of the heart.—The work done by the human heart was estimated by Gréhant[9] at 43,800 kilogrammetres in twenty-four hours, and this according to the mechanical equivalent of heat would give $\frac{43800}{424} = 103{,}000$ calories. Foster[10] calculates that the work done by the heart is nearly 60,000 kilogrammetres,

[1] Zuntz, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xii. S. 522.
[2] *Ibid.*, 1878, Bd. xviii. S. 255.
[3] *Ibid.*, 1878, Bd. xvi. S. 157.
[4] Rumpf, *ibid.*, 1884, Bd. xxxiii. S. 538; Pembrey, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894-1895, vol. xvii.
[5] "Goulstonian Lectures," *Lancet*, London, 1887, vol. i. p. 558.
[6] Béclard, *Arch. de méd. nav.*, Paris, 1861, pp. 24, 157, 257.
[7] Richet, *Compt. rend. Soc. de biol.*, Paris, 1892, p. 896.
[8] *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xlv. S. 625; and 1890, Bd. xlvi. S. 189.
[9] "Phys. Méd.," 1869, p. 229.
[10] "Text-Book of Physiology," 1891, 5th edition, pt. 1, p. 254.

Waller[1] estimates it at 20,000 kilogrammetres, and Nicolls[2] at 54,000 kilogrammetres.

The production of heat in glands.—Glands are the seat of active chemical changes, accompanied by a production of heat, but during activity their blood supply is augmented, and the increased temperature arising from this cause often masks the heat produced by the activity of the glands.

The *submaxillary gland* is an instance in which the activity of the tissue is accompanied by a greatly increased blood flow. Ludwig and Spiess[3] found by the thermo-electric method that the submaxillary saliva of a dog was 1° to 1°·5 warmer than the blood in the carotid artery. Bernard[4] ligatured the blood vessels of the gland, and found that stimulation of the chorda tympani still produced a slight rise in temperature, whereas excitation of the sympathetic produced a slight fall. The temperature in degrees is not stated, but Bernard brings these observations forward as additional arguments in favour of frigorific nerves. Morat[5] states that he has been able to confirm Bernard's results; Heidenhain,[6] on the other hand, observed a rise in temperature when the sympathetic was stimulated. Recently, Bayliss and Hill[7] have carefully investigated the question of the formation of heat in salivary glands; they used both the thermo-electric method and Geissler's thermometers. Their results led them to the following conclusions:—The temperature of the gland and tissues around it is almost as high as that of the aortic blood; the saliva is not warmer than the gland and tissues around the duct, and no formation of heat can be directly determined in the submaxillary gland by any known method of measuring variations in temperature. On stimulation of the chorda tympani, the temperature of the saliva never rose higher than the temperature of the aortic blood. No doubt the gland produces more heat during activity, but, on account of the small size of the gland, and the rapid circulation, the difference cannot be shown.

The intestines and liver.—According to Bernard,[8] the blood coming from the intestines is raised in temperature during digestion, the temperature of the blood in the portal vein being two- or three-tenths of a degree warmer than that of the abdominal aorta. Bernard also found that the liver was the warmest organ in the body, that the blood of the hepatic vein was higher than that of the portal vein, and showed a still further increase during digestion.

Stimulation of the splanchnic, or of the vagi nerves, produces no calorific or frigorific effect in the temperature of the liver.[9]

[1] "Human Physiology," 1893, 2nd edition, p. 75.

[2] *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 407.

[3] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1857, Bd. xxv. S. 584; Ludwig, *Wien. med. Wchnschr.*, 1860, Nos. 28 and 29.

[4] "Leçons sur la chaleur animale," 1876, p. 428.

[5] *Arch. de physiol. norm. et path.*, Paris, 1893, tome xxv. p. 285.

[6] *Stud. d. physiol. Inst. zu Breslau*, Leipzig, 1868, Bd. iv.

[7] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 351.

[8] "Leçons sur la chaleur animale," 1876, p. 190. See also this article, p. 809; Braune, *Virchow's Archiv*, 1860, Bd. xix. S. 470, 491.

[9] Waymouth Reid, "Proc. Phys. Soc.," *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii.

The Measurement of Heat Production.

The amount of heat produced by an animal can be determined by the measurement of the heat given off, and also by an estimation of the heat value of the chemical changes taking place in the body. The most exact method is that which embraces both of these determinations. Numerous attempts have been made to construct suitable calorimeters, but it is only within the last few years that exact methods have been devised.

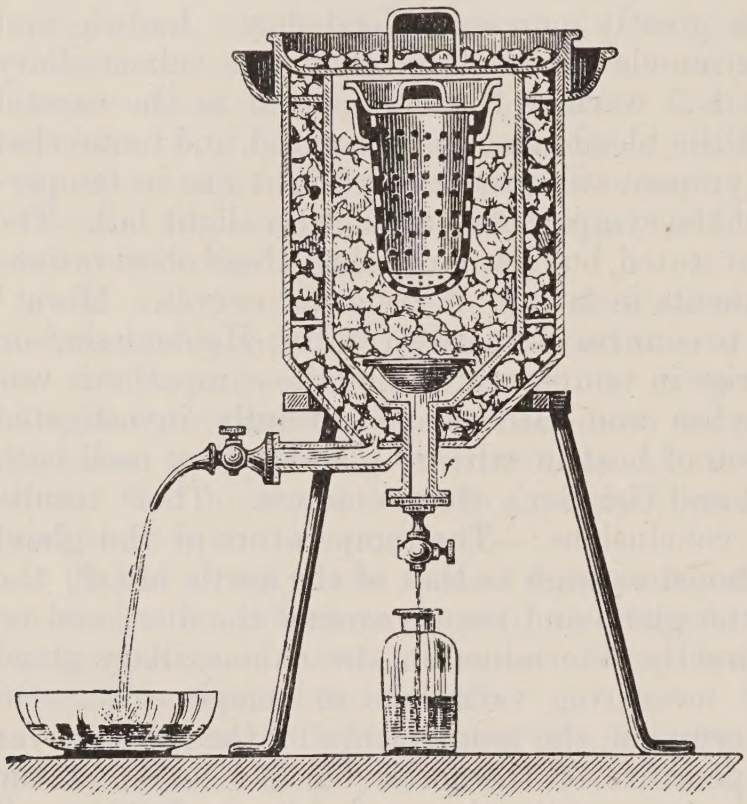


Fig. 81.—Diagram of ice calorimeter.

Calorimeters.[1]—In 1780, Lavoisier and Laplace [2] employed the ice calorimeter, in which the heat produced by the animal is estimated from the amount of ice liquefied. The construction of this calorimeter is shown in the accompanying diagram. (Fig. 81).

Important results were obtained by the use of this method, but they were not an exact measure of the heat produced by a normal animal. The exposure to such a low temperature causes an abnormal loss and production of heat, and it is impossible to rapidly and completely collect the water formed by the melting of the ice.

Crawford,[3] in 1788, introduced the water calorimeter, and indicated the precautions necessary to obtain accuracy. The method was improved by Dulong and Despretz.

Although this calorimeter was a great advance upon the ice calorimeter, yet it has been found by numerous observers to be unreliable. It is impossible, even by careful mixing, to obtain the exact heat of the water, for strata of different temperatures are formed, and thus errors easily arise. Further, the water responds very slowly to any change in the production of heat by the animal. This method was used by Dulong [4] and Despretz,[5] and has been again brought into use by Wood, Reichert, and others.[6]

The air calorimeter appears to have been first used by Scharling[7] in 1849, and

[1] A list of researches in which different kinds of calorimeters have been used, will be found in the paper by Haldane, Hale White, and Washbourn, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 124.

[2] *Hist. Acad. roy. d. sc.*, Paris, 1780, p. 355.

[3] "Experiments and Observations on Animal Heat," London, 1788, 2nd edition.

[4] *Ann. de chim. et phys.*, Paris, 1843, Sér. 3, tome i. p. 440 ; *Compt. rend. Acad. d. sc.*, Paris, tome xviii. p. 327.

[5] *Ann. de chim. et phys.*, Paris, 1824, Sér. 2, tome xxvi. p. 337.

[6] Wood, "Fever," *Smithson. Contrib. Knowl.*, Washington, 1880 ; Reichert, *Univ. Med. Mag.*, Philadelphia, 1890, vol. ii. p. 173.

[7] *Journ. f. prakt. Chem.*, Leipzig, 1849, Bd. xlviii. S. 435.

the most exact of the modern methods are modifications of this.[1] D'Arsonval,[2] in 1886, introduced the differential air calorimeter, which has this great advantage, that the loss of heat by conduction and radiation from the calorimeter containing the animal is compensated by a similar loss from a dummy calorimeter of similar size and construction. This method has been employed, and still further modified, by Rosenthal[3] and Rubner,[4] but it will suffice here to describe only the latest form, that introduced by Haldane, Hale White, and Washbourn.[5] In this calorimeter (Fig. 85) the heat produced by the animal in one chamber is balanced by the heat given off by a hydrogen flame burning in another similar chamber. The amount of hydrogen burnt is estimated, and, knowing the heat of combustion of hydrogen, one can calculate the calories produced by the quantity of hydrogen used in the experiment; this number of calories is equal to those given off by the animal. The calorimeter is so arranged that at the same time it serves as a respiratory apparatus, and the determination of the intake of oxygen and output of carbon dioxide checks the result of the calorimetric observation.

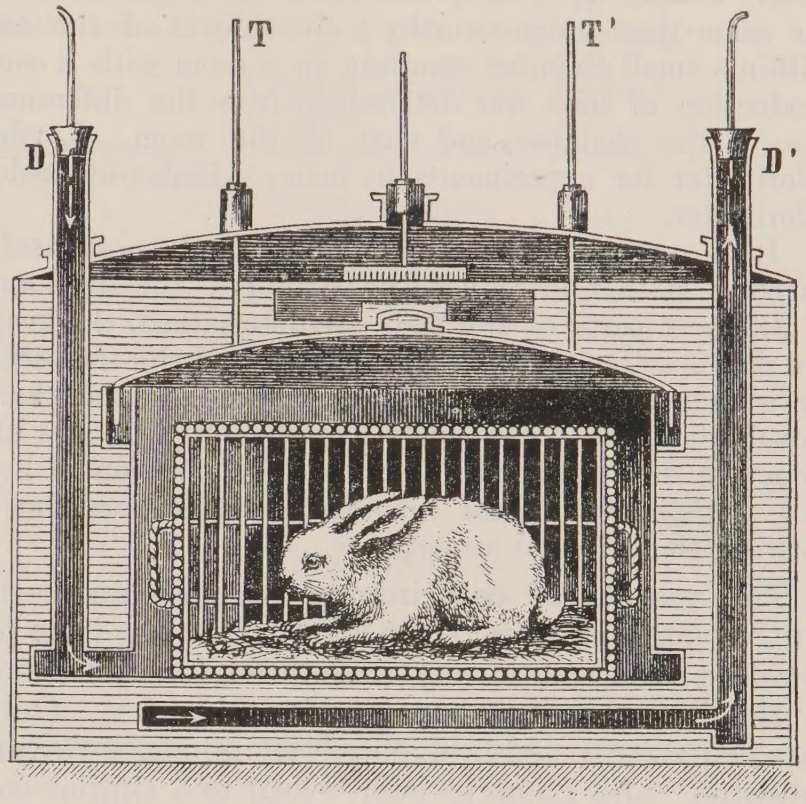


FIG. 82.—Diagram of Dulong's water calorimeter.

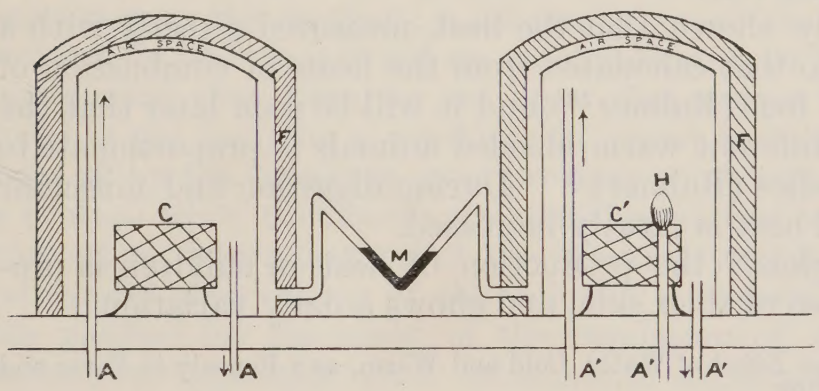


FIG. 83.—Diagram of air calorimeter (Haldane, Hale White, and Washbourn).

F. Layer of felt.
C. Cage.
A. Tubes for ventilation.
H. Hydrogen flame.
M. Manometer.

[1] Rosenthal, *Arch. f. Physiol.*, Leipzig, 1878, S. 349; Richet, *Arch. de physiol. norm. et path.*, Paris, 1885, tome vi. p. 237; Mosso, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1890, Bd. xxvi. S. 316.

[2] *Journ. de l'anat. et physiol. etc.*, Paris, 1886, tome xxii.

[3] *Arch. f. Physiol.*, Leipzig, 1888, S. 1.

[4] "Calorimetrische Methodik," Marburg, 1891; *Beitr. z. Physiol. Carl Ludwig z. s. 70 Geburtst.*, Leipzig, 1887; *Ztschr. f. Biol.*, München, 1893–94, Bd. xxx. S. 92.

[5] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 123; Hale White, Croonian Lectures, *Lancet*, London, 1897, vol. ii.; and *Brit. Med. Journ.*, London, 1897, vol. ii. p. 11.

For experiments on man, Currie,[1] and afterwards Liebermeister[2] and others, used a bath as a water calorimeter; this method is liable to many sources of error. Scharling,[3] Vogel,[4] and Hirn[5] used a method which was simple, but at the same time untrustworthy; the subject of the experiment was enclosed within a small chamber standing in a room with a constant temperature; the production of heat was determined from the difference between the temperature of the chamber and that of the room. Leyden employed a partial calorimeter for experiments in man; a limb was enclosed in a suitable water calorimeter.

It is probable that the simplest and most useful method for clinical purposes is that introduced by Waller[6]; the deep and surface temperatures of different parts of the body are determined, the evaporation of water from the skin is estimated by a hygrometer, and the temperature of the surrounding air is noted. If the calorimetric value of the thermometer scale be previously determined on a surface giving off heat at a known rate, it is possible from the data obtained to calculate the emission of heat. The apparatus, in fact, constitutes a heat manometer measuring the temperature difference between the skin and atmosphere.

The results of calorimetric experiments.—Lavoisier[7] and Crawford[8] concluded from their results that the heat produced by an animal could be almost entirely accounted for by the combustion represented by the discharge of carbon dioxide and water. Dulong[9] and Despretz's[10] data, when corrected by Liebig,[11] Helmholtz,[12] Gavarret,[13] Ludwig,[14] Milne Edwards,[15] and Liebermeister,[16] lead to a similar conclusion, but since the more exact experiments of Rubner and others, they have had only a historical interest.[17]

The table on p. 847 gives some of the more important results obtained by various observers.

It has been already shown that the heat, measured directly with a calorimeter, is equal to that calculated from the heats of combustion of the constituents of the food (Rubner[18]), and it will be seen later that the production of heat in different warm-blooded animals is proportionate to the surface of their bodies (Rubner).[19] During digestion and muscular work the production of heat is greatly increased.

According to Langlois,[20] the production of heat in children is proportionate to the surface of their skin, and shows a daily variation.

[1] "Medical Reports on the Effect of Water, Cold and Warm, as a Remedy in Fever and other Diseases," Liverpool, 1798.

[2] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1860, S. 520, 589; 1861, S. 28; "Handbuch der Path. u. Therap. des Fiebers," 1875, S. 142.

[3] *Journ. f. prakt. Chem.*, Leipzig, 1849, Bd. xlviii. S. 435.

[4] *Arch. d. Ver. f. wissensch. Heilk.*, Leipzig, 1864, S. 442.

[5] "Recherches sur l'équivalent mécanique de la chaleur," Paris, 1858.

[6] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894, vol. xv.

[7] *Hist. Acad. roy. d. sc.*, Paris, 1780, p. 355.

[8] "Experiments and Observations on Animal Heat," 1788, 2nd edition.

[9] *Ann. d. chim. et phys.*, Paris, 1843, Sér. 3, tome i. p. 440.

[10] *Ibid.*, 1824, Sér. 2, tome xxvi. p. 337.

[11] "Thierchemie." S. 28.

[12] "Encyclop. Wörterb. d. med. Wissensch.," 1846, Bd. xxxv. S. 523.

[13] "De la chaleur produite par les êtres vivants," 1855, p. 219.

[14] "Lehrbuch d. Physiol.," 1861, Aufl. 2, Bd. ii. S. 739.

[15] "Leçons sur la physiologie," 1863, tome viii. p. 23.

[16] "Handbuch der Path. u. Therap. des Fiebers," 1875, S. 134.

[17] For a discussion of these results see Rosenthal, Hermann's "Handbuch," Bd. iv. Th. 2, S. 358.

[18] *Ztschr. f. Biol.*, München, 1894, Bd. xxx. S. 135. This article, pp. 833–37.

[19] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 535. This article, p. 853.

[20] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, S. 237.

Heat Produced.	Animal.	Remarks.	Observer.
132,000 cal. per hour.	Adult man.	At rest.	Scharling.[1]
140,000 ,, ,,	,, ,,	,,	Hirn.[2]
99,000 ,, ,,	,, ,,	Partial calorimeter used.	Leyden.[3]
21,000 cal. per hour.	Dog weighing 5350–5450 grms.	One hour after food.	Senator.[4]
12,630 ,, ,,	,,	Twenty-six hours after food.	...
10,900 ,, ,,	,,	About forty-nine hours after food.	...
2,500 cal. per hour and per kilo.	Dogs.	About thirty-six hours after food. Mean of experiments on six dogs.	...
5,920 cal. per hour and per kilo.	Pigeon.	Normal.	Corin and Van Beneden.[5]
6,000 ,, ,,	,,	After removal of cerebral hemispheres.	...

The respiratory exchange as a measure of heat production.—The heat of the body has been shown to be due to processes of combustion occurring in the tissues. The respiratory exchange is a measure of this combustion, and hence a determination of the intake of oxygen and the output of carbon dioxide is a measure, although not a perfectly accurate one, of the heat produced. There is, however, one source of inaccuracy in this method; a determination of the respiratory exchange during a limited time is not an exact indication of the combustion occurring during that time, for we know that oxygen may be taken up and stored in the body for a considerable period, and carbon dioxide may be given off by the breaking up of previous combinations; in fact, may still be evolved when the tissues are receiving no free oxygen. Nevertheless, consecutive determinations of the respiratory exchange for long periods, and careful observations of the animal's temperature, form a most valuable method for the study of the regulation of temperature by heat production, especially since calorimetric experiments are more tedious, difficult, and more open to accidental sources of error.

In the case of warm-blooded animals, a fall in external temperature increases, a rise diminishes the intake of oxygen and the output of carbon dioxide. Crawford[6] and Lavoisier[7] came to this conclusion not only on theoretical grounds, because they believed that animal heat was due to combustion, but from the results of direct experiment.

[1] *Journ. f. prakt. Chem.*, Leipzig, 1849, Bd. xlviii. S. 435.

[2] "Recherches sur l'équivalent mécanique de la chaleur," Paris, 1858; "Exposition analytique et expérimentale de la théorie mécanique de la chaleur," Paris, 1875, 3e édition, tome i. p. 27.

[3] *Deutsches Arch. f. klin. Med.*, Leipzig, 1869, Bd. v. S. 273.

[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1871; *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1872, S. i.; 1874, S. 18; "Untersuch. ueber den fieberhaften Process und seine Behandlung," Berlin, 1873, S. 30.

[5] *Arch. de biol.*, Gand, 1887, tome vii. p. 276.

[6] "Experiments and Observations on Animal Heat," 1788, 2nd edition.

[7] *Hist. Acad. roy. d. sc.*, Paris, 1780, p. 407.

Numerous observations have been made, chiefly by Pflüger and his pupils, upon the effect of changes in external temperature upon the respiratory exchange of animals in normal and abnormal conditions. The results of some of the most important experiments will now be given :[1] —

Temperature of Air.	Intake of Oxygen.	Output of Carbon Dioxide.	$\frac{CO_2}{O_2}$	Animal.	Observer.
4°·4	...	210·7 grms. in 6 hours.	...	Man weighing 71 kilos.	Voit.[2]
6°·5	...	206·0 ,,	...		
9°·0	...	192·0 ,,	...		
14°·3	...	155·1 ,,	...		
23°·7	...	164·8 ,,	...		
26°·7	...	160·0 ,,	...		
30°·0	...	170·6 ,,	...		
7°·3	1496·66 c.c. per hour and kilo.	1203·44 c.c.	0·80	Guinea-pig.	Colasanti.[3]
16°·9	1086·8 ,,	937·01 ,,	0·86		
3°·64	1856·8 ,,	1564·8 ,,	0·83	Guinea-pig.	Finkler.[4]
26°·21	1118·5 ,,	1057·4 ,,	0·94		
−5°·5	17·48 grms. in 6 hours	19·83 ,,	...	Cat weighing about 2·5 kilos.	Herzog Carl Theodor.[5]
2°·0	15·79 ,,	17·87 ,,	...		
12°·3	17·71 ,,	17·63 ,,	...		
20°·1	12·78 ,,	14·34 ,,	...		
29°·6	13·91 ,,	13·12 ,,	...		
−9°·0	...	3016 c.c. per hour and kilo.	...	Pigeon.	Corin and Van Beneden.[6]
17°·5	...	1141 ,,	...		

It will be seen from the above table that the respiratory exchange decreases with a rise in external temperature, until a point about 35° is reached, when an increase in the metabolism occurs.[7] The response to a change in temperature is, in the case of small mammals, almost immediate.[8] Thus, within two minutes of a change from 30° to 18°, a mouse increased its output of carbon dioxide by 74 per cent. ; within one minute of a change from 33°·25 to 17°·5 the increase was 60 per cent. The response to an increase in temperature does not take place so quickly ; thus, within two minutes of a rise from 18° to 34°·5, the decrease in the output of carbon dioxide was 18 per cent. ; within one minute of a rise from 17° to 32°, the decrease was 5 per cent. The power of maintaining a constant mean temperature is readily tested in this manner, as the following example will show (Pembrey) :—

[1] See also the preceding article on "Chemistry of Respiration."
[2] *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 57.
[3] *Arch. f. d. ges Physiol.*, Bonn, 1877, Bd. xiv. S. 92.
[4] *Ibid.*, 1877, Bd. xv. S. 603.
[5] *Ztschr. f. Biol.*, München, 1878, Bd. xiv. S. 51.
[6] *Arch de biol.*, Gand, 1887, tome vii. p. 274.
[7] See also Page, *Journ. Physiol.*, Cambridge and London, vol. ii. p. 228 ; and "Chemistry of Respiration," this Text-book, vol. i. p. 712.
[8] Pembrey, *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 401. See also "Chemistry of Respiration," this Text-book, vol. i.

Consecutive Periods of Thirty Minutes.

CO_2 in Decimilligrammes.	External Temperature.	Remarks.
1055	11°·0	Mouse shivering and active, face and ears pale.
957	11°·0	Mouse less active, ears pale.
518	31°·5	Mouse quiet, sweating, ears flushed.
262	32°·5	Mouse sprawled out, sweating, apparently asleep.
815	11°·0	Mouse wakes up, becomes very active, ears pale.
683	11°·0	Mouse quiet, ears pale.

The Production of Heat in Cold-Blooded Animals.

One of the most characteristic phenomena of life is an exchange of material, an oxidation which results in the production of heat. In the lowest forms of life, both vegetable and animal, a certain amount of heat is produced. Numerous experiments have shown that this is so, although, owing to the cooling effect of evaporation from the surface of the body, the heat produced may be masked by the excessive loss; the temperature of a frog may be lower than that of the air, notwithstanding that the animal is constantly producing heat.

It is unnecessary to give here an account of the temperature of plants,[1] but, in addition to the facts already stated,[2] further details must be brought forward concerning the production of heat in the lower animals. Hunter[3] found that the temperature of earth-worms, slugs, and leeches might be a degree above that of their surroundings; a carp had a temperature of 20°·6, a viper one of 20°, when that of the surroundings was 18°·6 and 14°·4 respectively.

In bees, even in winter, the capacity for producing heat has already been shown to be very great. Next in point of interest is the fact, to which attention was first drawn by Valenciennes,[4] that pythons, when coiled round their eggs during incubation, maintain a temperature even 20° above that of the surrounding air. The following are some of the results obtained by Sclater,[5] who compared the temperature of a female python with that of the non-incubating male, which was kept in the same compartment of the reptile house:—

Dates.	Temperature of Air in Den.	Temperature of Male.	Temperature of Female.
Feb. 12	14°·8	On surface, 21°·2	22°·8
		Between folds, 23°·8	27°·5
March 2	15°·6	On surface, 22°·0	28°·9
		Between folds, 24°·4	35°·6

[1] See on this subject Dutrochet, *Ann. d. sc. nat.*, Paris (Botanique) 1840, Sér. 2, tome xiii. pp. 5 and 65; Gavarret, "De la chaleur produite par les êtres vivants," Paris, 1855, p. 516; Sachs, "Physiology of Plants," p. 404; Vines, "Physiology of Plants"; Van Tieghem, "Traité de botanique," Paris, 1891, tome i. It is interesting to notice that an abnormal rise of temperature, fever in fact, has been observed in the tissues around a wound in a plant.—*Annals of Botany*, 1897.

[2] This article, p. 792.

[3] "Works," Palmer's edition, London, 1837, vol. iv. p. 131 *et seq.*

[4] *Compt. rend. Acad. d. sc.*, Paris, 1841, tome xiii. p. 126.

[5] *Proc. Zool. Soc. London*, 1862, p. 365.

Forbes[1] made similar observations, and found, as the average temperatures between the folds of the body, 30° and 31°·7 in the case of the male and female respectively; the maximum was 32°·1 for the male, and 33°·8 for the female. The greatest difference between the temperature of the air and the surface of the snake was 4°·6 in the male, and 5°·3 in the female; between the air and the coils of the snake, 6°·4 in the male, and 9°·3 in the female. It is worthy of note that the female took no food and little exercise for many weeks before and during incubation.

In some fishes a temperature several degrees above that of the water has been observed. Thus Davy[2] found the temperature of deep-seated muscles of the bonito (*Thynnus pelamys*) to be 37°·2, when that of the sea was 26°·9. The tunny (*Thynnus thynnus*) is said to have a similar high temperature.

The embryo of the chick must be looked upon as a cold-blooded animal, for it responds to changes of temperature in a similar manner,[3] yet even at an early stage the production of heat within its tissues can be shown to be considerable. Thus Bärensprung[4] found that the temperature of an egg on the fourth day of incubation was ·6 above that of a dead egg and ·8 above that of the incubator.

The Regulation of Loss of Heat.

An animal may lose heat in various ways—by direct conduction and radiation from the skin, by evaporation of sweat, by the warming of air during respiration and by evaporation from the different parts of the respiratory system, by raising cold food and drink to the temperature of its body, and by the discharge of urine and fæces. Loss of heat is controlled chiefly by the skin and the lungs.

The distribution of the loss of heat by an adult man in twenty-four hours has been estimated by various observers as follows:—

	Vierordt.	Helmholtz.	Ludwig.
1	1·8 % by urine and fæces .. = 47,500 calories,	= 2·6 %	2·1 %
2	3·5 % by expired air .. .. = 84,500 ,,	= 2·6 %	7·2 %
3	7·2 % by evaporation from lungs = 182,120 ,,	=14·7 % 2,732,000 calories.	10·3 % 2,706,076 calories.
4	14·5 % ,, ,, ,, skin = 364,120 ,,		
5	73·0 % by radiation and conduction from skin .. .. .. 1,791,820 ,,	=80·1 %	78·5 %
	2,500,000		

Loss of heat by the skin—Radiation and conduction. — The amount of heat lost by radiation and conduction is, within certain limits, in proportion to the difference in the temperature of the body and of its surroundings; the warmer the skin and the colder the surroundings, the greater will be the loss of heat. The heat of the skin is controlled by the cutaneous circulation, and this in turn is regulated by the central nervous system. The general result is that the cutaneous blood vessels are contracted, the circulation is smaller, and the skin pale and cold, when the external temperature is low; on the other hand, the vessels dilate, the circulation becomes greater and the skin red and warm, when the temperature of the surroundings is high.

[1] *Proc. Zool. Soc. London*, 1881, p. 960.

[2] "Researches," London, 1839, vol. i. p. 219.

[3] Pembrey, Gordon, and Warren, *Journ. Physiol.*, Cambridge and London, 1894–95, vol. xvii. p. 331.

[4] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1851, S. 131.

Thus it happens that the animal can diminish or increase its loss of heat according to its needs.

Other conditions, however, play an important part in this regulation. In the case of man the epidermis and the subcutaneous fat are bad conductors; and, by means of clothing,[1] the greater part of the body is so protected that it is in contact not with the external air, but with a fairly stationary layer of air, with a temperature from 24° to 30°. In other warm-blooded animals protection is afforded by the fur or feathers, which prevent loss of heat not only by their thickness and slight power of conduction, but by enclosing strata of more or less stationary warm air. The more stationary the air the less the loss of heat, for the body becomes surrounded with a layer of air having a temperature intermediate between that of the body and of the atmosphere. Thus, during Parry's expedition to the Polar seas, the sailors found that they could better bear a cold which would freeze mercury (−40°), when the air was perfectly calm, than a temperature of −12°·2 when there was a wind.[2] The men of Franklin's expedition had the same experience.[3] Further, the capacity of dry air to take up heat is much less than that of moist air; hence it happens that in dry, calm air several degrees below zero, much less sensation of cold may be felt than in moist air with a temperature a few degrees above the freezing point.

In the whale, seal, and walrus, the thick epidermis and the large amount of subcutaneous fat so perfectly prevent excessive loss of heat, that their high temperature can be maintained in the Arctic seas. Greyhounds, on the other hand, feel even moderately cold weather very quickly, for, as the result of selective breeding, they have little fur and hardly any subcutaneous fat.[4] Vierordt[5] calculated that an adult man lost 1,791,820 calories, or 73 per cent. of the total loss of heat, by radiation and conduction from the skin in twenty-four hours. Masje,[6] from experiments made with a thermoscope, constructed on the principle of Langley's bolometer, concludes that the heat lost by radiation from the skin of an adult man, weighing 82 kilos. and with a surface of 20,000 square cms., is 1,700,000 calories in twenty-four hours. Similar experiments have also been made by Stewart.[7]

Evaporation.—Benjamin Franklin[8] observed, during the hot weather at Philadelphia in 1750, that his temperature remained normal, although the external temperature was 37°·8 in the shade. He attributed this result to the cooling effect of the evaporation of sweat. This was proved by Blagden[9] during his experiments upon the effect of extreme heat on the body. When the air was moist, the temperature of the body rose; whereas in dry air, heated to 126°, the temperature did not rise above the normal. Into the heated room two jars of water were brought, and a layer of oil was placed on the surface of the water in one, with the

[1] Schuster, *Arch. f. Hyg.*, München u. Leipzig, 1888, Bd. viii. S. 1; Rubner, *ibid.*, 1890, Bd. xi. S. 255.

[2] "Journal of a Second Voyage to the Arctic Regions."

[3] Franklin, "Journey to the Polar Sea, 1819–1822," 2nd edition, vol. ii. pp. 27, 28. See also Ross, "Narrative of a Second Voyage in Search of a North-West Passage," London, 1835, pp. 285, 287, 297.

[4] Bergmann, "Göttinger Studien," 1847, Abth. 1, S. 595.

[5] "Grundriss der Physiol. des Menschen."

[6] *Virchow's Archiv*, 1887, Bd. cvii. S. 17, 267.

[7] *Stud. Physiol. Lab. Owens Coll.*, Manchester, 1891, vol. i. p. 100.

[8] "Experiments and Observations on Electricity," London, 1769, p. 366.

[9] Blagden, *Phil. Trans.*, London, 1775, vol. lxv. pp. 111 and 484.

result that it quickly boiled, owing to the absence of evaporation; whereas the water in the other jar rose to 60°, but did not boil, evaporation taking place freely from the surface and thus cooling the water. Upon the loss of heat by evaporation Crawford,[1] in 1781, made some interesting experiments upon frogs; he compared the rates of warming of a dead and a living frog by exposure to warm air and to warm water, and found that the temperature of the former rose more rapidly than that of the latter.

In another experiment he found that, when the air was 25°, the skin of a living frog was 20°, the stomach 21°·4; when the water was 16°·1, the skin of a living frog was 16°·2, the stomach 19°·2. It is to be noted that when the frog was kept in water its nose was above the surface, so that it might breathe; in this way heat might be lost by evaporation from the lungs. Crawford, however, concluded from his experiments that the cooling was not solely due to evaporation, and that animals had the power of "producing cold."

In 1810, Delaroche[2] published some instructive experiments, similar to those of Blagden, to show the effect of evaporation. He placed an alcarraza[3] full of water at 35°, and a rabbit whose temperature was 39°·7, in a stove heated to 45°; the temperature of the rabbit gradually rose to 43°·8, while that of the alcarraza fell to 31°·4, and remained stationary. In the second experiment he placed a frog and two pieces of moist sponge in a stove heated to 36°·5, and found at the end of an hour that the frog's temperature was stationary at 28°·2, and that of the pieces of sponge at 27°·9 and 27°·6. Delaroche contests the results of Crawford's experiments on frogs, and maintains that these animals quickly take the temperature of the water in which they are placed, and that in this respect there is no difference between a dead and a living frog.

According to the calculations of Vierordt and Ludwig, from 10 to 20 per cent. of the total daily loss of heat in an adult man is due to evaporation from the skin and respiratory tract. Further details on the discharge of water from the skin and lungs are given in another part of this work.[4] The following values for the discharge of moisture from various parts of the human skin were observed by Waller:[5]—

Palm of hand .	24	mgrms.	per 20 sq. cm.	(per 10 minutes)
Sole of foot .	14	,,	,,	
Forehead .	12	,,	,,	
Cheek . .	6	,,	,,	
Axilla . .	10	,,	,,	
Popliteal space	10	,,	,,	
Forearm . .	5	,,	,,	
Leg . .	5	,,	,,	

External temperature, 20°.

The influence of the size of the body upon the regulation of temperature.—The importance of the relation between the surface of the body and its mass, in respect to the loss and production of heat, was first pointed out by Bergmann.[6] The bigger an animal the greater the ratio of

[1] Crawford, *Phil. Trans.*, London, 1781, vol. lxxi. p. 485.

[2] Delaroche, *Journ. de phys.*, Paris, 1810, tome lxxi. pp. 294–296.

[3] A porous jar used for keeping water cool in hot climates. See also Edwards, "De l'influence des agens physiques sur la vie," p. 84.

[4] "Chemistry of Respiration," this Text-book, vol. i. p. 711.

[5] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. For further observations see Hale White, Croonian Lectures, *Lancet*, London, 1897, June 19 and 26, and *Brit. Med. Journ.*, London, 1897, vol. i. p. 1654 *et seq.*

[6] "Göttinger Studien," 1847, Abth. 1, S. 595.

its volume or weight to its surface, for weight or volume increases as the cube, surface increases as the square. Thus, if the dimensions of a body be increased from 1 to 2, the surface increases from 1 to 4, and the cubic content from 1 to 8. A small animal, therefore, has a far greater surface in relation to its weight than a large animal, and if it is to maintain its temperature at a similar height, it must have either special means for preventing an excessive loss of heat or a more rapid production of heat. Both of these means are employed, and so effective are they, that the temperature of the smallest mammals and birds is often higher than that of the biggest. A mouse has a thicker covering of hair, and, relatively to its weight, a greater production of heat, than a horse. Further, as remarked by Bergmann, the smaller animals need a relatively greater supply of food.

The large animals living in the tropics, such as the elephant and hippopotamus, are often remarkable for the small amount of hair upon the body and for their love of bathing, whereby the loss of heat is favoured. The largest mammals, the whales, are able by means of their enormous size and special layers of fat to resist the cold of the Arctic seas, and maintain a temperature equal to that of mammals living in the tropics. Water-fowl, especially those which inhabit cold regions, are noted for the protection afforded against cold by their down and feathers.

These indications from the natural history of animals are fully confirmed by experimental observations. The determinations made by Letellier,[1] and by Regnault and Reiset,[2] show that the intake of oxygen and the output of carbon dioxide are relatively greater in small than in large animals[3]; starvation is more rapidly fatal to small than to large animals,[4] for during life they consume a relatively larger quantity of proteid.[5] Further, Rubner[6] has determined the heat production of dogs of different size, and finds that the smaller animals produce relatively more heat in proportion to their weight than the larger animals, and that the heat production is proportional to the surface of the body. The following table gives some of these results:—

Weight.	Surface.	Surface per Kilo. Weight.	Heat Production per Kilo. per Day. Air=15°.	Heat Production per Square Metre of Surface.
Kilo.	Sq. Cm.	Sq. Cm.	Kilo-cal.	Kilo-cal.
31·2	10,750	344	35·68	1036
18·2	7662	421	46·20	1097
9·6	5286	550	65·16	1183
6·5	3724	573	66·07	1153
3·19	2423	726	88·07	1212

Similar results have been obtained by Langlois[7] in the case of children.

[1] *Ann. de chim. et phys.*, Paris, Sér. 3, tome xiii. p. 478.
[2] *Ibid.*, 1849, tome xxvi. p. 299; 1863, tome lxix. p. 129.
[3] See article "Chemistry of Respiration," this Text-book, vol. i. pp. 706–8.
[4] Chossat, "Recherches expérimentales sur l'inanition," Paris, 1843.
[5] Voit, Hermann's "Handbuch," Bd. vi. S. 88.
[6] *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 535.
[7] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, S. 237.

Rubner calculates that the tissues of a rat produce five and one-third times, the tissues of a sparrow thirteen times, as much heat as the same weight of tissue in a man.

The Influence of the Nervous System upon the Regulation of Temperature.

The nervous system exercises a control on both of the factors concerned in the regulation of temperature; upon the loss of heat by means of the vasomotor system, which regulates the amount of blood in the deep and superficial parts of the body, and by the respiratory centre which controls the frequency and depth of respiration; upon the production of heat through the nerves which control the activity of the tissues, chiefly the muscles. The control is of the nature of a reflex, and the sensory nerves of the skin and muscles are probably the most usual lines of the afferent impulses. The most important nervous centres are the vasomotor and the respiratory, but in addition to these and the so-called "motor" centres some physiologists maintain that special "heat centres" exist in the brain.

Vasomotor control of temperature.—The blood distributed to the body comes from the heart, where the temperature is, with the exception of the liver and a few other internal parts, the highest in the body; this warm blood is carried to the extremities and the surface of the body, where the temperature is lower. Now, three zones may, as Rosenthal[1] has pointed out, be recognised—an internal warm zone, an intermediate temperate zone, and an external cool zone; the first is represented by the deep organs and tissues, the second by the more superficial parts, and the third by the skin and subcutaneous tissue. Under ordinary circumstances the temperature will decrease from within outwards, for the most important seats of chemical change and heat production are situated within the first two zones, and the loss of heat is greatest from the surface of the skin. The blood circulating in the vessels distributes the warm blood of the interior to the superficial parts, and carries back cooler blood from the surface to the interior. The difference, therefore, in temperature between the interior and the surface will depend upon the rapidity and the quantity of the blood circulating through the different zones of the body; this distribution is regulated by the central nervous system through the vaso-constrictor and vaso-dilator nerves. The vasomotor nerves have their centre in the medulla oblongata, and probably subordinate ones in the spinal cord; the distribution, however, of these centres and nerves is discussed elsewhere; here they will be considered merely as part of the nervous mechanism which regulates temperature.

When the cutaneous and subcutaneous vessels are constricted, the quantity of blood distributed to the skin is diminished, the difference between the temperature of the surface of the body and its surroundings is less, and consequently less heat is lost. This condition is brought about by external cold, and thus the heat of the body is economised and its normal temperature is maintained, or may, under certain circumstances, be raised, for it has already been shown that the first effect of a cold bath is to raise the temperature in the axilla and rectum. On the other hand, exposure to warmth causes a dilatation of the cutaneous

[1] Hermann's "Handbuch," 1882, Bd. iv. Th. 2, S. 381.

vessels, the difference between the temperature of the skin and its surroundings is increased, and likewise the loss of heat. Thus the first effect of a warm bath may be a fall in the temperature of the internal parts. The loss of heat by this flushing of the skin with hot blood and by sweating may be very great, as shown by the rapid fall in temperature during the sweating state of ague or the crisis of pneumonia.

These changes in the calibre of the vessels can be brought about reflexly, not only by sensations of heat and cold but by those of pain; further, emotions can effect these changes, as in the blushing of excitement or shame, and the pallor of fright or anger; in fact, emotions may in different individuals have opposite effects upon the vascularity of the skin.

An impression conveyed by the sensory nerves of one part of the body can influence the calibre of the vessels, not only on the same side but also on the opposite side. Thus, Brown-Séquard and Tholozan,[1] found that plunging one hand in warm water raised the temperature of the opposite hand also. Waller,[2] however, has failed to confirm this.

The explanation of the part played by the vasomotor nerves in the regulation of temperature is not so simple as may appear from a first consideration, for the problem is complicated by the fact that an increase or decrease in the vascularity of the skin is accompanied by a similar change in the production of sweat; further, it is possible that the alterations in vascularity may affect the metabolism of the tissues. Upon this latter point there has been considerable discussion. The first and most important experiment in this connection is that of Bernard,[3] who found that section of the cervical sympathetic caused a dilatation of the blood vessels and a rise of temperature in the ear of the same side. The enlargement of the blood vessels results in a greater and more rapid flow of blood through the ear, and this would naturally raise the temperature of the part. Bernard, however, did not look upon this explanation as complete; he held that the nervous system regulated not only the circulation but also the production of heat in the tissues, for he states, among other arguments, that section of the cervical sympathetic, after previous ligature of the veins of the ear, still caused a rise of temperature. According to Bernard, the nerve was both vaso-constrictor and frigorific. It was to be expected, however, that this view would be contested, for although a certain amount of heat would be produced in the ear, as in the metabolism of all tissues, yet that amount would be small, for the cartilage and other tissues of the ear are not the seats of an active exchange of material.

Numerous experimenters[4] have decided against Bernard's theory, and have attributed the changes in the temperature of the ear to alterations

[1] *Journ. de l'anat. et physiol. etc.*, Paris, 1858, tome i. p. 497.

[2] Note communicated to the writer.

[3] "Leçons sur la physiologie et la pathologie du systeme nerveux," 1858, tome ii. p. 490; "Leçons sur la chaleur animale," 1876, p. 297.

[4] Brown-Séquard, *Med. Exam.*, Philadelphia, 1852, p. 489, and 1853, p. 9; Budgé, *Compt. rend. Acad. d. sc.*, Paris, tome xxvi. p. 337; *Ztschr. v. d. Verein f. Heilk. in Preussen*, 1853, Bd. xxii. S. 149; Waller, *Compt. rend. Acad. d. sc.*, Paris, 1854, tome xxxvi. p. 378; De Ruyter, "De actione atropæ belladonnæ," Diss., 1853; Schiff, "Untersuch. z. Physiol. des Nervensystems," 1855, Bd. i. S. 124; *Allg. Wien. med. Ztg.*, 1859, S. 318; Kussmaul and Tenner, *Untersuch. z. Naturl. d. Mensch. u. d. Thiere*, 1855, Bd. i. S. 92; Callenfels, *Ztschr. f. rat. Med.*, 1858, Bd. vii. S. 157; Jacobson and Landre, *Nederl. Tijdschr. v. Geneesk.*, Amsterdam, Bd. i. Heft 3; Donders, Wünderlich's "Medical Thermometry," p. 148; Bayliss and Hill, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 351.

in the blood supply alone. The difference in the temperature of the two ears, after section of the cervical sympathetic on one side, may be even as great as 12° or 16°, but it is proportionate to the difference in the quantity of blood (Schiff). If the two subclavians and the carotid on the same side as the divided sympathetic are ligatured, the temperature of the ear falls below the normal, owing to the want of collateral circulation; on the other hand, the temperature of the ear can be raised by ligature of the subclavians without section of the sympathetic nerve; this is due to the increased pressure of blood in the carotid artery (Kussmaul and Tenner). The ears of a rabbit are to be looked upon as part of the mechanism for regulating temperature by the varying quantity of blood exposed; section of one sympathetic causes a fall in the temperature of the ear of the opposite side (Jacobson and Landre).

In addition to the vasomotor nerves of the skin, it is important to remember that the vasomotor nerves to the respiratory tract and lungs may play an important but subordinate part in the regulation of the loss of heat.[1] The importance of this method of regulation without doubt varies in different animals, and is greater in those with a thick coat of fur, as in the dog, who, when he is too hot, pants with open mouth and lolling tongue. This rapid respiration, 150–200 per minute in heated dogs, has been specially studied by Ackermann,[2] Goldstein,[3] and Riegel;[4] more recently, Richet[5] has shown that a dog gives off from its respiratory tract, every hour, about 1 grm. of water for every kilo. of its body weight, when the external temperature is moderate, but when exposed to a hot sun it discharges ten times as much moisture and increases its respirations from 28 to 230 per minute. Any cause which prevents a dog from breathing rapidly and freely, such as a tight muzzle, causes a rise of two or three degrees in the animal's temperature.

The temperature of the body after damage or section of the spinal cord.—An examination of the numerous observations made upon the influence of injury or section of the spinal cord shows at first sight much confusion and apparent contradiction in the results. In the majority of cases, however, the results can be harmonised by taking into account the numerous factors of secondary import. In the first place, the experiments are only strictly comparable when they are performed upon similar animals under similar conditions. Thus the effect will vary according to the level of the injury or section of the spinal cord; a section high up in the cord will involve a more extensive paralysis than one low down, and the more extensive the paralysis the smaller the production, and the greater the loss of heat, owing to the dilated cutaneous vessels. A section above the splanchnic area will obviously have a greater effect than one below that area; a section high up in the cord will interfere with the movements of respiration, whereas one low down will have comparatively little effect. Again, an animal with only the lower extremities and part of the trunk paralysed, may be able to maintain its temperature by greater variations in the production and loss of heat in the parts still under control. The size of the animal is important, for the bigger the animal the smaller is its surface in relation to its

[1] Bradford and Dean, *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 34. Here an account of previous work on the subject will be found.

[2] *Deutsches Arch. f. klin. Med.*, Leipzig, Bd. ii. S. 361.

[3] Inaug. Abhandlung, *Verhandl. d. phys.-med. Gesellsch. in Würzburg*, 1871, S. 156.

[4] *Virchow's Archiv*, 1874, Bd. lxi. S. 396.

[5] *Compt. rend. Soc. de biol.*, Paris, 1887, p. 482.

mass, and thus the loss of heat due to vasomotor paralysis is less serious than in a small animal. Animals also differ in their method of regulation; some, as in the case of man, have a well-developed vasomotor system for the cutaneous surface, which is so slightly protected by natural covering; others, as in the case of dogs, have a thick fur, and regulate their temperature chiefly by variations in the production of heat and in the loss of heat from the respiratory tract. The distribution and part played by the sweat glands varies greatly, as shown by a comparison of men and horses with dogs and cats. It is to be noticed further, in this respect, that marked differences exist even in individuals of the same race and variety; thus, some men and horses sweat much more readily and profusely than others.

In addition to the above factors, it is necessary to consider the external conditions under which the injured animal finds itself. The external temperature greatly modifies the part played by the loss of heat from the paralysed parts. Most animals adopt a different posture, according to their need of heat or cold; thus a heated dog, rabbit, or mouse lies with extended trunk and limbs, whereas the same animal when it is cold, coils or huddles itself together. It is almost unnecessary to point out that a paralysed animal could not assume these instinctive postures. A normal rabbit tied down in an extended position loses an abnormal quantity of heat, and its temperature falls, and in some cases the body is so greatly cooled that death results.[1]

The above facts must therefore be borne in mind during any examination of the effects of section or injury of the spinal cord.

Attention was first drawn to the influence of the nervous system upon temperature, by the experiments and clinical observations of Benjamin Brodie.[2] He found that, after the head of an animal was cut off, or the cord divided high up in the cervical region, the circulation of the blood still continued when artificial respiration was performed, but the temperature fell even more quickly than in a dead animal. This Brodie correctly attributed to the great loss of heat from the circulating blood, for if the circulation was stopped by ligature of the heart, the fall of temperature was much retarded. It was also found that woorara (curari) and essential oil of almonds, by suspending the action of the central nervous system, also caused a fall in temperature. Brodie further compared the discharge of carbon dioxide by normal rabbits with that of rabbits with the brain removed or poisoned by woorara or the essential oil of almonds; he states that the same quantity of carbon dioxide is formed in each of these cases, and therefore that the heat production is not due to chemical change but to nervous action. This conclusion is not warranted by the results of the determinations of the respiratory exchange, and the results themselves are not comparable, for, even when it was possible, the experiments were not made upon the same animals.

The work of Brodie led to numerous experiments and discussions on this subject by Chossat,[3] Hale,[4] Legallois,[5] Wilson Philip,[6] Hastings,[7]

[1] Legallois, *Ann. de chim. et phys.*, Paris, 1817, Sér. 2, tome iv. p. 21.

[2] *Phil. Trans.*, London, 1811, vol. ci. p. 36; 1812, vol. cii. p. 378; *Med.-Chir. Trans.*, London, 1837, vol. xx. p. 146.

[3] *Deutsches Arch. f. d. Physiol.*, Halle, 1822, Bd. vii. S. 282.

[4] *London Med. and Phys. Journ.*, vol. xxii.

[5] *Ann. de chim. et phys.*, Paris, 1817, Sér. 2, tome iv.

[6] "Experimental Inquiry into the Laws of the Vital Functions," London, 1818, 2nd edition, p. 197 *et seq.*

[7] *Quart. Journ. Sc. Lit. and Arts*, London, 1823, vol. xiv. p. 96.

and C. J. B. Williams.[1] The results on some points confirmed, on others contradicted, Brodie's conclusions. Wilson Philip found that artificial respiration caused a fall in the temperature of intact animals, and that a slow ventilation prevented the temperature of the brainless animal from falling as quickly as that of a dead animal. Hastings obtained similar results, and Williams confirmed the observations of Wilson Philip, that the temperature of a brainless animal might even be slightly raised by artificial respiration. Legallois carried out a very complete series of experiments upon the subject, and came to the following conclusions: that a brainless animal upon which artificial respiration was performed suffered a reduction of temperature, but it was from one to three degrees less than in a dead animal; that in cooling through a certain number of degrees it parted with more heat than a dead animal; that inflation of the lungs of normal animals lowered their temperature,[2] and if the ventilation were continued for a long time they might die of cold; and, finally, that a fall in temperature might be produced by any condition which constrained or impeded the respiration.

Tscheschichin[3] found that section of the spinal cord between the third and fourth cervical vertebræ caused the temperature of a rabbit to fall from 38°·9 to 32°·1. This he attributed to the increased loss of heat from the paralysed cutaneous vessels, and to diminished production of heat; the higher the section, the more extensive the paralysis of the blood vessels, and the greater the loss of heat; stimulation of the peripheral end of the cord caused contraction of the blood vessels, and the loss of heat was less.

In rabbits, section of the spinal cord at the commencement of the dorsal region caused the rectal temperature to fall from 40° to 24° in five hours (Bernard).[4] In guinea-pigs, section of the upper dorsal region produced a progressive fall in the rectal temperature from 38°·9 to 16° in twenty-four hours, when the animal died (Pochoy).[5]

Fischer[6] found a rise of 0°·5 to 1°·7 in the temperature of dogs and rabbits after complete section of the cervical portion of the spinal cord, but no rise when the operation was performed in the dorsal or lumbar regions. He concluded that an inhibitory centre for heat existed in the cervical region of the cord. A series of experiments were made by Naunyn and Quincke[7] upon the effect of crushing the spinal cord. They selected dogs of large size, and with thick fur, in order to diminish the importance of the loss of heat. They found that, after the cord was crushed at the level of the sixth cervical vertebra, the rectal temperature fell, unless the excessive loss of heat due to vasomotor paralysis was prevented by a fairly high external temperature; if the air was warm, the temperature rose two or three degrees, and even higher after death. These observers concluded that there were nerve fibres which, passing from the brain to the spinal cord, inhibited the production of heat[8]; and that, after section, the production as well as the loss of heat were

[1] "Observations on the Changes produced in the Blood in the course of its Circulation," London, 1835.

[2] See also Fawcett and Hale White, *Journ. Physiol.*, Cambridge and London, 1897, vol. xxi. p. 435.

[3] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1866, S. 151.

[4] "Leçons sur la chaleur animale," 1876, p. 161.

[5] Thèse, Paris, 1870.

[6] *Centralbl. f. d. med. Wissensch.*, Berlin, 1869, No. 17.

[7] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1869, S. 174, 521.

[8] See also Ott and Collmar, *Journ. Nerv. and Ment. Dis.*, N.Y., 1887, p. 428.

increased, and if the augmentation of the latter was not excessive, the temperature of the body rose. On the other hand, Riegel[1] found that the production of heat was diminished, and he explains the rise of temperature in Naunyn and Quincke's cases as due to absence of the rapid breathing whereby normal dogs regulate their temperature. Further, Schroff[2] found a rise in the temperature of dogs when they were kept in a warm chamber after opening of the spinal canal, without damage to the spinal cord.

Rosenthal[3] repeated Naunyn and Quincke's experiments, but never found any rise of temperature, unless the animals were kept in a chamber warmed to 32°. If the section was made lower down in the cord, more muscles remained under the control of the animal, and by the contraction of these muscles more heat was produced, and the temperature raised when the external air was warm. Rosenthal further points out that it is probable that septic fever was the cause of the rise of temperature in some of Naunyn and Quincke's dogs.

Pflüger's[4] experiments upon the respiratory exchange of rabbits, after section of the spinal cord in the lower cervical region, show that such an animal is comparable to a cold-blooded animal; a rise in external temperature increases, a fall diminishes, the metabolism and the temperature of the animal. The same result is even more markedly shown in the case of a smaller animal. Thus the following figures show the effect of sudden changes in the external temperature upon the output of carbon dioxide of a mouse before and after section of the spinal cord in the lower cervical region:[5]—

Before Section of Cord. Consecutive Periods of 15 Minutes.			Three Hours after Section of Cord. Consecutive Periods of 15 Minutes.		
CO_2 in Decimilligrammes.	Temperature of Water Bath.	Remarks.	CO_2 in Decimilligrammes.	Temperature of Water Bath.	Remarks.
391	25°·0	Mouse very quiet.	222	22°·0	Mouse quiet.
372	24°·0	,, ,, ,,	229	22°·0	,, ,,
558	12°·5	Mouse active.	250	11°·75	Mouse moves its forelimbs very actively.
572	12°·5	,, ,,	158	11°·75	Mouse quiet.

We may conclude, therefore, that in animals the general effect of section of the spinal cord in the lower cervical region is a fall in the temperature of the body, due to a reduction in the metabolism of the paralysed muscles, and to excessive loss of heat consequent upon the vasomotor paralysis. The exceptional cases appear to be due to a high external temperature, and to interference with the rate of respiration, which in dogs plays an important part in the cooling of the body.

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1872, Bd. v. S. 629.

[2] *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, Wien, Bd. lxxiii. Abth. 3, S. 141.

[3] "Zur Kenntniss d. Wärmeregulierung bei den warmblütigen Thieren," S. 35; Hermann's "Handbuch," Bd. iv. Th. 2, S. 437.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1878, Bd. xviii. S. 321.

[5] Pembrey, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894–1895, vol. xvii.

An examination of the cases of crushed spinal cord in man shows discordant results, in some cases a marked rise, in others a fall in the temperature of the body. The following table gives the chief data in some of the cases recorded:—

Sex and Age.	Seat of Injury.	Temperature.	Remarks.	Observer.
M.	Crush at level of fifth and sixth cervical vertebræ.	43°·9 (111° F.), between scrotum and thigh.	Diaphragmatic breathing; respiration slow and irregular; pulse weak; countenance livid; death twenty-four hours after accident.	Brodie.[1]
M., 28.	Crush at level of fifth and sixth cervical vertebræ.	35° (95° F.), rectal.	Paralysis extended to 1 in. above nipple line; patient complained of feeling cold.	Hutchinson.[2]
		35° (95° F.), rectal and axillary just before death.	Death on fifth day; no injury to other parts of body.	
M., 53.	Crush at level of fifth cervical vertebra.	Below 35° (95°), 4 P.M., first day.	Complete paralysis of all limbs; diaphragmatic breathing; skin cold and dry; pulse 72.	,,
		Below 35° (95°), 6.30 P.M., first day.	Skin cold and clammy; patient complains of feeling cold.	
		Below 35° (95°), 11.30 P.M., first day.	Pulse 50.	
		36°·8 (98°·2), morning, second day.	Skin warm and dry; pulse 64.	
		36°·9 (98°·4), evening, second day.	Skin hot and dry.	
		38°·9 (102°), afternoon, third day.	Death.	
M., 36.	Crush at level of seventh cervical vertebra.	36°·9 (98°·4), sixth hour after accident.	Diaphragmatic breathing; imperfect paralysis of arms; patient felt very cold.	,,
		38°·6 (101°·5), third day.	Skin of natural warmth; pulse 72.	
		38°·0 (100°·4), fourth day.	.....	
		39°·0 (102°·2), fifth day.	Pulse 99.	
		37°·45 (99°·4), sixth day.	Face mottled; pulse 84; death.	
M.	Crush at level of fifth cervical vertebra.	40°·0 (104°·0), first day, axilla.	Slight power of moving shoulders and upper arms; skin flushed and very hot.	Churchill.[3]
		40°·3 (104°·5), second day.	Pulse 96, regular, quiet.	
		38°·9 (102°·0), third day.	.....	

[*Continued on next page.*

[1] *Med. Chir.-Trans.*, London, 1837, p. 146.
[2] *Lancet*, London, 1875, vol. i. pp. 713, 747.
[3] Quoted from Hutchinson, *loc. cit.*

Sex and Age.	Seat of Injury.	Temperature.	Remarks.	Observer.
M.— *contd.*	Crush at level of fifth cervical vertebra.	41°·1 (106°), fourth day.	40°·2 (104°·4) on foot; patient died soon after in a violent spasm, and a quarter of an hour after death, temperature in axilla = 43°·3 (110°).	Churchill.[1]
M., 48.	Crush at level of sixth cervical vertebra; fracture of skull.	33°·5 (92°·3), on admission to hospital. 27°·6 (81°·7), forty-eight hours later.	Temperature, taken in both rectum and axilla, fell steadily until death. Death.	Wagstaffe.[1]
M., 22.	Crush between sixth and seventh cervical vertebræ.	33°·9 (93°·0), on admission. 27°·8 (82°·0), a few hours before death.	Patient drowsy and prostrate. Death about forty-eight hours after accident.	Le Gros Clarke.[1]
M., 39.	Crush at level of fourth and fifth cervical vertebræ.	34°·5 (94°·1), four hours after the accident. 36°·5 (97°·7), eighth hour. 41°·6 (106°·9), 7 A.M., second day. 42°·4 (108°·3), 1 P.M., second day.	.... Death at 2.5 P.M. on second day; post-mortem rise to 42°·9 (109°·2) in ten minutes.	Billroth.[2]
M., 34.	Crush at level of fifth and sixth cervical vertebræ.	37°·6 (99°·7), five hours after accident. 40°·9 (105°·6), twelfth hour. 42°·1 (107°·8), fifteenth hour. 43°·6 (110°·5), nineteenth hour, eleventh minute. 43°·2 (109°·7), nineteenth hour, thirty-fifth minute.	Complete paralysis of trunk and limbs; diaphragmatic breathing. .... Death.	Frerichs.[3]

These discordant results have to be explained. It is worse than useless to say that the effects are due to the removal of the regulating influence of "heat centres" in the brain, centres whose very existence is problematical. Observations upon the deep and surface temperature, and upon the amount of moisture given off by the skin, are needed to show whether the changes in temperature are due to disturbance in the production or in the loss of heat, or more probably in both. The data upon these points are insufficient, but, recently, such observations have been made by Pembrey,[4] in the case of two

[1] Quoted from Hutchinson, *loc. cit.*

[2] *Arch. f. klin. Chir.*, Berlin, 1868, Bd. ix. S. 161.

[3] Recorded by Lorain, "De la temperature du corps humain," tome i. p. 500.

[4] "Proc. Physiol. Soc.," *Journ. Physiol.*. Cambridge and London, 1897, vol. xxi.; *Brit. Med. Journ.*, London, 1897, vol. ii. p. 883.

patients suffering from traumatic section of the spinal cord. The general result is a subnormal temperature so long as the patient's condition is not complicated by other internal or external disturbance. The subnormal temperatures are due to excessive loss and diminished production of heat, owing to the vasomotor and motor paralysis. The section of the spinal cord high up in the cervical region abolishes the power of regulating temperature. When the patient is exposed even to moderate cold, his temperature falls owing to the increased loss of heat and to the diminished production of heat. On the other hand, if the weather be hot and the patient be too well covered with bedclothes, his temperature rises, and may reach a dangerous height, owing to the diminished loss and the increased production of heat in the body. In the paralysed man the production of heat rises and falls *with* the external temperature. In the case of the high temperatures there are several factors which may play an important part; the paralysed parts soon cease to sweat; in fact, Horsley has shown that, by the use of pilocarpine, it is possible to localise the level of the injury to the cord. The respiration is hampered, it is only diaphragmatic; the ventilation of the lungs is therefore imperfect, and less heat is lost by the cooling of the inspired air, and by the evaporation of water from the respiratory tract to saturate the expired air with moisture. Further, the warmer the paralysed tissues the greater is their metabolism and production of heat.

It naturally follows that, in cases of section of the spinal cord in the dorsal or lumbar regions, the regulation of temperature is less disturbed.

The influence of the brain upon the regulation of temperature.—It is impossible to state concisely and dogmatically the influence of the brain upon the temperature of the body. With our present knowledge it is only permissible to review the chief results obtained by various observers, and to draw some provisional conclusions.

In 1866, Tscheschichin[1] published the results of experiments, which showed that a section between the medulla oblongata and the pons Varolii caused a rise in the temperature of rabbits. In one case the rectal temperature rose in two hours from 39°·4 to 42°·6, and at the same time there was a corresponding increase in the rate of the pulse and respiration. On the other hand, section of the spinal cord between the third and fourth cervical vertebræ caused, in another rabbit, a fall in temperature from 38°·9 to 32°·1. From these experiments Tscheschichin concluded that a moderator centre exists in the brain, and prevents the excessive activity of an augmentor heat centre in the medulla oblongata. Lewizky[2] repeated but could not confirm these experiments; he observed a steady fall in temperature after the operation. The subject was then taken up, under the guidance of Heidenhain, by Bruck and Günther,[3] who, working upon rabbits, obtained positive results in eleven, negative in twelve cases. They found in one case a rise from 39°·31 to 42°·5 in the rectal temperature, two or three hours after the operation. These observers further found that simple puncture with a probe between the pons and medulla was more effectual than section, and they noticed that the rise in temperature occurred not only in the interior, but also in the peripheral parts of the body, a fact which indicates that the rise is due to increased production of heat. Bruck and Günther do not agree with Tscheschichin's view of a moderator centre, for they point out that the results can be produced by electrical

[1] *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1866, S. 151.
[2] *Virchow's Archiv*, 1869, Bd. xlvii. S. 357.
[3] *Arch. f. d. ges. Physiol.*, Bonn, 1870, Bd. iii. S. 578.

stimulation as well as by puncture of that portion of the nervous system, and are probably due to traumatic stimulation. It is to be noted that irregular muscular movements were observed in many of the cases.

Schreiber,[1] from the results of experiments performed upon rabbits, came to the conclusion that a rise of temperature followed injury to all parts of the pons, to the pedunculi cerebri, cerebellum, and cerebrum, when the animal was protected by a covering of wool or flannel against excessive loss of heat; injury between the medulla oblongata and the pons always caused a rise in temperature. In most cases, however, the rise in temperature was very small, and the experiments were often complicated by spasms of the muscles.

Observations upon the production of heat, as determined by a calorimeter, and also upon the animal's temperature after lesions of various parts of the central nervous system, were made by Wood.[2] Section of the spinal cord above the origin of the splanchnic nerves produced an increase in the loss but a decrease in the production of heat; on the other hand, section between the medulla oblongata and the pons caused an increase in both the production and loss of heat, and for this reason Wood supported the view of Tscheschichin, that a moderator centre exists in or above the pons.

Eulenberg and Landois[3] found that in dogs destruction of a portion of the cortex of the brain in the neighbourhood of the sulcus cruciatus caused a rise of temperature, which was most marked on the side of the body opposite to the lesion; they looked upon this effect as due to vasomotor disturbance. These results were confirmed by Hitzig[4] and Wood, but on rabbits Küssner[5] and H. Rosenthal[6] obtained negative results.

Injury to the front of the brain was found by Richet[7] to produce a rise of temperature, and Ott[8] obtained a similar result by injury to the corpus striatum; this observation was confirmed by Girard,[9] Baginsky and Lehmann.[10] In 1885, Aronsohn and Sachs[11] published the results of an important series of experiments upon rabbits; they found that puncture with a probe, the greatest thickness of which was 3 mm., had no effect upon the temperature of the body when the operation was performed upon the front part of the cerebral hemispheres, but a puncture passing through the median side of the corpus striatum near the nodus cursorius of Nothnagel caused, within a few hours, a rise of temperature which persisted for two or three days. The rise varied from 1°·7 to 2°·4, and could also be produced by electrical stimulation of the corpus striatum. Control experiments showed that the injury to

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1874, Bd. viii. S. 576.

[2] "Fever, a Study in Morbid and Normal Physiology," *Smithson. Contrib. Knowl.*, Washington, 1880.

[3] *Centralbl. f. d. med. Wissensch.*, Berlin, 1876, No. 15; *Virchow's Archiv*, 1876, Bd. lxviii. S. 245.

[4] *Centralbl. f. d. med. Wissensch.*, Berlin, 1876, No. 18.

[5] *Ibid.*, 1877, No. 45.

[6] "Einfluss des Grosshirns auf des Korperwärme," Diss., Berlin, 1877.

[7] *Compt. rend. Soc. de biol.*, Paris, 29th March 1884, p. 189; *Compt. rend. Acad. d. sc.*, Paris, 31st March 1884; *Arch. de physiol. norm. et path.*, Paris, tome vi.

[8] *Journ. Nerv. and Ment. Dis.*, N.Y., 1884, Nos. 7 and 8; 1887, p. 152; 1888, p. 551; *Therap. Gaz.*, Detroit, 1887; *Brain*, London, 1889.

[9] *Arch. de physiol. norm. et path.*, Paris, 1886, tome viii.

[10] *Virchow's Archiv*, 1886, Bd. cvi. S. 258.

[11] *Arch. f. d. ges. Physiol.*, Bonn, 1885, Bd. xxxvii. S. 232.

the cortex during the performance of the puncture did not cause any rise of temperature. The high internal temperature after puncture of the corpus striatum was accompanied by an increase in the temperature of the skin, and by an increase in the respiratory exchange, and in the discharge of nitrogen in the urine. The mean result of the determinations of the respiratory exchange was as follows:—

	Rectal Temperature.	Oxygen.	Carbon Dioxide.
		in c.c. at 0° C., 760 m.m. per Kilo. and Hour.	
Before puncture	38°·5	664°·0	626°·7
After puncture	39°·8	749°·7	715°·8

Aronsohn and Sachs conclude that the rise in temperature after the puncture is due to increased production of heat, and increased metabolism, arising from the stimulation of the corpus striatum.

These experiments have been repeated and extended by Hale White,[1] who found no rise in the temperature of rabbits after lesions of the white matter of the cerebrum, but an almost constant effect after injury of the corpus striatum and optic thalamus. In the case of lesions of the corpus striatum, the rectal temperature rose to 41°·6 in two cases, to 41°·1 in eleven cases, and to 40° in eighteen; while in three cases there was a slight rise, and in two a fall in temperature. The average rise was 1°·7, and was attained within four to sixteen and a half hours after the operations, and persisted for about sixty-two hours. After lesions of the optic thalamus, the average rise of temperature was 1°·4. Hale White concludes that the corpus striatum and the optic thalamus can modify the temperature of the body, and that they do not work directly through the vasomotor system. No increase in the discharge of carbon dioxide was observed in rabbits after damage to the corpus striatum.[2]

Several cases of a rise in temperature in man after a hæmorrhage into the corpus striatum have been recorded.[3]

Recently Tangl[4] has observed the effect of puncture through the anterior part of the optic thalamus in horses. In one case the temperature rose to 40°·8 within twenty-four hours, in another to 40°·4 within sixteen hours of the operation, and in two other cases there was no effect. The temperature remained only for a short time at the above height, and then fell.

Fredericq[5] found that removal of the cerebral hemispheres in pigeons caused practically no difference in the daily curve of their rectal temperature. This observation has been confirmed by Corin and Van Beneden,[6] who have, in addition, shown that the pigeons without their cerebral hemispheres produce the same amount of carbon dioxide and heat

[1] *Journ. Physiol.*, Cambridge and London, 1890, vol. xi. p. 1.

[2] Hale White, Croonian Lectures, *Lancet*, London, 1894, July 10, and *Brit. Med. Journ.*, London, 1897, vol. ii. p. 71.

[3] Bourneville, Ferrier, J. H. Bryant, Hale White; references given by Hale White, *Brit. Med. Journ.*, London, 1894, 17th Nov.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lxi. S. 559.

[5] *Arch. de biol.*, Gand, 1882, tome iii. p. 747.

[6] *Ibid.*, 1889, tome vii. p. 265.

as do normal pigeons. The rapid rise in temperature which occurs when a hibernating marmot awakes, is not prevented by removal of the cerebral hemispheres.

An impartial examination of the above evidence leads to the verdict that the existence of the so-called "heat centres" in the brain has not been proved. In the first place, the results, even in the hands of the same experimenter, are inconsistent; some observers obtain exactly opposite effects from apparently similar lesions. Further, in many cases rabbits have been used for these experiments, and it is notorious that their temperature is liable to considerable variations during operative procedures. Even if the existence of these centres be granted, even if it be allowed that after puncture there is an increase in metabolism and in the production of heat, it by no means follows that the centres are special centres for the regulation of temperature, and give off "thermic nerves."

It seems more probable that the mechanism of heat regulation has the same cerebral representation as the voluntary muscles. In the lower warm-blooded animals the representation of these in the cerebral cortex is not well developed, and it has likewise been shown that the removal of the cortex in them has little or no effect upon the regulation of temperature.

The Development of the Power of Maintaining a Constant Temperature.

In the cold-blooded animals there are traces of the power of maintaining a constant temperature, as shown by the high temperature which a female python is able to maintain for many weeks when she is incubating her eggs. This instance is the more remarkable because during that time the python takes no food or exercise. Further instances have already been mentioned in the case of bees, and some species of fish.

It is possible to trace in the warm-blooded animals the gradual development of this power of regulation. Thus, during the development of a chick there is first a stage in which the embryo responds to changes of temperature in a similar manner to that of a cold-blooded animal; then a stage of transition in which there is a regulation for moderate changes of temperature; and finally, when a chick is hatched, the power of regulation resembles that of a warm-blooded animal.[1] In 1824, Edwards[2] pointed out that young mammals and birds may be divided into two classes, the warm-blooded and the cold-blooded, according as they are, or are not, able to maintain their temperature when removed from the warmth of the parents. The difference lies in the relative development of the two classes—active young animals covered with fur or feathers, as in the case of the guinea-pig and chick, belong to the former class; while young animals born naked, blind, and helpless, belong to the cold-blooded group. The inability to maintain a constant temperature is due to diminished production of heat on exposure, and only secondarily to excessive loss of heat. It has recently been shown that the chick and guinea-pig can at birth regulate their production of heat, that young cold-blooded mammals and birds are able to regulate only for moderate changes of external temperature; for, when exposed to cold, their temperature and

[1] Pembrey, Gordon, and Warren, *Journ. Physiol.*, Cambridge and London, 1894–95, vol. xvii. p. 331.

[2] "De l'influence des agens physiques sur la vie," 1824.

production of carbon dioxide fall, and they resemble cold-blooded animals. About the fifteenth day after birth, they respond to a fall in the temperature of their surroundings with increased muscular activity and output of carbon dioxide, and thus maintain their temperature (Pembrey [1]).

In the lowest mammals the temperature is much lower than in the higher members of the group. Thus the temperature of the *Echidna hystrix* is 27°·5, and that of the Ornithorhynchus, 24°·8.[2]

Further, a hibernating mammal is an instance of an animal at one time warm-blooded and at another time cold-blooded, and its power of regulation is in many respects similar to that of an immature mammal.[3] Additional proofs of the gradual development of the power of maintaining a constant temperature are found in the unstable temperature of infants and animals. In premature and weak infants the power is imperfect, and the temperature is below the normal.[4] It is in man that the perfection of this power is reached; he of all animals has the most constant temperature under extreme differences of external heat and cold.

It is impossible with our present knowledge to state what are the structural differences which accompany the development of the power of regulation. This much we may say: the power appears to be associated chiefly with the control of the nervous system over the skeletal muscles and those of the blood vessels. An anæsthetic, or curari, or section of the spinal cord reduces a warm-blooded animal to a cold-blooded condition; its temperature and production of carbon dioxide vary with, and in the same direction as, the temperature of its surroundings. In a hibernating animal the fall of temperature is accompanied by greatly diminished activity of the muscular and nervous systems; and the sudden rise in temperature, when the animal awakes from its torpidity, is marked by a sudden increase in the discharge of carbon dioxide and in muscular activity. Those young mammals and birds which are born with well-developed control over their muscular system, are able to regulate their temperature even at birth, whereas those born in a helpless condition do not attain this power until a week or two after birth, at a time when their power of co-ordination is much increased.

The Temperature of the Body after Death.

After death the temperature of the body generally falls, the loss of heat varying according to the difference between the temperature of the corpse and that of its surroundings; another important factor is the surface of the body in relation to its mass, for the corpse of an infant or of a wasted subject cools more rapidly than that of a well-developed adult.[5] In some cases, however, a rise of temperature is observed in the corpse, especially when death has resulted from tetanus, acute rheumatism, typhoid fever, small-pox, cholera, or injuries to the brain and spinal cord. A few of the cases recorded are given in the following table:—

[1] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 363.

[2] Miklоucho Maclay, *Proc. Linn. Soc. New South Wales*, 1883, vol. viii. p. 425; vol. ix. p. 1205; Semon, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 229.

[3] Pembrey and Hale White, *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. 477.

[4] Crombie, *Indian Ann. Med. Sc.*, Calcutta, 1873, vol. xvi. p. 597; Raudnitz, *Ztschr. f. Biol.*, München, 1888, Bd. xxiv. S. 423.

[5] Taylor and Wilks, *Guy's Hosp. Rep.*, London, 1863, p. 184; observations on one hundred cases; Sutton, *Brit. Med. Journ.*, London, 1874, vol. i. p. 153; Bidder and Schmidt, "Die Verdäuungssafte und der Stoffwechsel," S. 323; Womack, *St. Barth. Hosp. Rep.*, London, 1887, vol. xxii. p. 193; Niderkorn, "De la rigidité cadaverique chez l'homme," Paris, 1872.

Temperature before Death.	Temperature after Death.	Disease.	Place of Observation.	Observer.
...	45° (3½ hrs. post-mortem)	Pyæmia	Left ventricle.	Davy.[1]
...	42°·2 (5½ hrs. post-mortem)	Sudden death, cause undetermined.	,,	,,
...	43°·75 (1 hour post-mortem)	Small-pox.	Axilla.	Simon.[2]
...	44°·5	,,	,,	,,
41°·1	44°·5	Sunstroke.	,,	Levick.[3]
44°·75	45°·4 (57 min. post-mortem)	Tetanus.	,,	Wunderlich.[4]
...	41°·2	Cholera.	Rectum.	Mackenzie.[5]
42°·3	43°·2 (15 min. post-mortem)	Erysipelas.	Axilla.	Eulenburg.[6]
40°·4	42°·3 (20 min. post-mortem)	,,	,,	,,
...	41°·8	Sunstroke.		Thompson.[7]
36°·1	38°·3 (7½ min. post-mortem)	Apoplexy.	,,	De Haen.[8]
41°·6	43° (30 min. post-mortem)	Tetanus.	,,	Lehmann.[9]
43°·01	44°·03 (1 hour post-mortem)	Pyæmia.	Rectum.	Quincke and Brieger.[10]
42°·1	43°·4 (1 hour post-mortem)	Pneumonia delirium tremens.	,,	,,
41°·1	43°·3 (15 min. post-mortem)	Crush of spinal cord.	Axilla.	Churchill.[1]

The causes of this post-mortem rise in temperature have been investigated by various observers.[12] The most important factors are these. When the circulation and respiration cease at death, the normal loss of heat from these causes and from sweating also comes to an end, but the tissues live for a short time and produce heat even after the death of the organism as a whole. If this production of heat is greater than the loss of heat from the corpse, the temperature rises; if, on the other hand, it is less, then the effect is only to delay the fall of temperature. The next source of heat is in the muscles on the onset of rigidity; and, finally, when decomposition sets in, and this may after some diseases occur exceedingly rapidly, there is a further production of heat due to putrefaction. In some cases the temperature of a corpse does not fall to that of the atmosphere even in four or five days.[13]

[1] "Researches," London, 1839, vol. i. p. 228.
[2] *Ann. d. Char.-Krankenh. . . . zu Berlin*, 1865, Bd. xiii. Heft 2, S. 1.
[3] *Penn. Hosp. Rep.*, Philadelphia, 1868, vol. i. p. 369.
[4] *Arch. d. Heilk.*, Leipzig, 1861, Bd. ii. S. 547.
[5] *London Hosp. Rep.*, vol. iii. p. 454.
[6] *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, No. 5.
[7] *Brit. Med. Journ.*, London, 9th July 1870.
[8] Quoted from Valentin, *Deutsches Arch. f. klin. Med.*, Leipzig, 1869, S. 201.
[9] *Schmidt's Jahrb.*, Leipzig, 1868, Bd. cxxxix. S. 241.
[10] *Deutsches Arch. f. klin. Med.*, Leipzig, 1879, Bd. xxiv. S. 284.
[11] Churchill, quoted from Hutchinson, *Lancet*, London, 1875, vol. i. p. 713.
[12] Besides those above enumerated, the following may be mentioned:—Seume, Thesis, Leipzig, 1856; Erb, *Deutsches Arch. f. klin. Med.*, Leipzig, 1865; Thomas, *Arch. d. Heilk.*, Leipzig, 1868, Bd. ix. S. 17, 31; Goodhart, *Brit. Med. Journ.*, London, 1874, vol. i. p. 303; Huppert, *Arch. d. Heilk.*, Leipzig, 1867, Bd. viii. S. 321; Fick and Dybkowsky *Vrtljschr. d. naturf. Gesellsch. in Zurich*, 1867; Schiffer, *Centralbl. f. d. med. Wissensch.* Berlin, 1867, S. 849; *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1868, S. 442.
[13] The author is indebted to Drs. Haldane, Hale, White, and Waller for valuable suggestions on various points, dealt with both in this and in the preceding article.

METABOLISM.

By E. A. Schäfer.

Contents :—Introductory, p. 868—Balance of Nutrition, p. 871—Composition of Foodstuffs, p. 872—Heat Value of Foodstuffs, p. 874—Necessary Amount of Proteid, p. 875—Special Constituents of Diet, and their Effect on Metabolism, p. 878—Gelatin, p. 878—Carbohydrates, p. 880—Fats, p. 881—Inorganic Substances, p. 882—Metabolism in Inanition, p. 887—With purely Proteid Diet, p. 891—Relative Metabolic Activity of Tissues, p. 895—Nitrogenous Metabolism, p. 896—Influence of the Liver on Proteid Metabolism, p. 900—Influence of Muscular Activity on Proteid Metabolism, p. 911—Metabolism of Carbohydrates, p. 916—Glycogen formation, p. 919—Phloridzin Diabetes, p. 920—Glycogenesis, p. 922—Puncture Diabetes, p. 926—Action of Pancreas on Carbohydrate Metabolism, p. 927—Metabolism of Fat, p. 930—Source and Formation of Fat, p. 931—Action of Liver on Metabolism of Fat, p. 935.

Introductory.—The word "metabolism" has come into use in this country as the equivalent of the German word *Stoffwechsel*, which strictly means "exchange of material." The subject which it denotes embraces all that is known or conjectured regarding the changes which occur within the body in the materials of the food, or foodstuffs, and in the materials which compose the tissues and organs of the body itself, or bodystuffs. Generally, however, the *digestive* changes in the food are excluded from the scope of the expression. There is no special reason, other than that of convenience of description, why this should be the case, for the digestive changes in the food must, like all other chemical changes occurring within the body, influence the general conditions of the economy. The usual course will, however, be followed in this article, and I shall confine what I have to say to the changes that occur after the food is absorbed, in so far as they have not been already treated of in the articles in this work dealing with the chemistry of the urine and with the chemical processes of respiration and heat production, both of which subjects constitute essential parts of the whole subject of metabolism.

The metabolic changes which are undergone by the tissues must be of two kinds, which are opposite in nature.[1] For, on the one hand, the complex molecules which constitute living tissue or *bioplasm*,[2] are built

[1] Hering, "Vorgänge der lebenden Materie," Prag, 1888. A translation, by Miss F. A. Welby, of this extremely important and interesting article will be found in *Brain*, London, 1897, vol. xx. p. 232.

[2] I use the word *bioplasm* as a synonym for living substance, rather than *protoplasm*, because the latter word has come to have a definite *histological* rather than a *physiological* signification ; and, on the one hand, is used to include portions of cell substance which, for aught we know, may not be actually living matter, whilst, on the other hand, it does not include the living substance of the cell nucleus, which would be included in the expression "bioplasm."

up from non-living materials, furnished by the food; and, on the other hand, they are broken down into simpler substances, which pass away from the tissue into the blood, and ultimately from the body with the excreta, or, as in the case of secretory glands, directly into secretions. The building-up process, whereby fresh molecules of bioplasm are formed, has come to be spoken of as an anabolic change (*anabolism, assimilation*), and the breaking-down process as a katabolic change (*katabolism, dissimilation*). It is clear that these two processes will produce opposite effects upon the bioplasm, the one increasing and the other diminishing its bulk. But, on the other hand, it is conceivable that even within the same cell there may be, at the same time, both a building up or anabolic change proceeding, so that fresh molecules of bioplasm are being formed, and also a breaking-down or katabolic change, affecting molecules which have been formed previously, and the net result to the bulk of the tissue may be *nil*, provided that these two processes balance one another; that is to say, the bioplasm, although undergoing active metabolic changes, and furnishing products of its metabolism to the secretions or to the blood, is not altered in amount (autonomous equilibrium). But although both processes are occurring simultaneously, they nevertheless do not exactly balance one another, there will be as the net result either a gain or loss of bioplasm, *i.e.* the bioplasm of the cell will increase or diminish in amount. If every cell were entirely composed of bioplasm, this would evidently involve an increase or diminution in the bulk of the cell itself. But besides the actual bioplasm, all cells contain in a variable proportion products of the activity of their bioplasm; "formed material," in the sense of Lionel Beale, as distinguished from "formative matter." If these products remain within the cell, it may, in spite of the fact that katabolic processes are proceeding within it more actively than anabolic processes, still increase in bulk, even to a very large extent, but without any corresponding increase, indeed even with an actual diminution, of its bioplasm.

Various circumstances may determine the general direction of the metabolism of a cell, whether upward in the direction of increased anabolism with increase of bioplasm, or downward in the direction of increased katabolism with decrease of bioplasm. One such circumstance is undoubtedly the amount and nature of the pabulum supplied to the cell. Another is to be found in the general physical conditions of the environment, such as variations of temperature, supply of water and of oxygen, and the like. And in the case of many animal cells we may well suppose (and indeed the point may be said to have been determined for specific instances) that impulses derived from the nervous system may set up respectively, according to their nature, or the nervous channel along which they are conveyed, metabolic changes in either an anabolic or a katabolic direction. Thus it has been suggested by Gaskell that the heart nerves act upon its muscular substance, so as to produce respectively anabolic changes (vagus fibres, inhibitory impulses) and katabolic changes (sympathetic fibres, augmentor and accelerator impulses), accompanied by diminished activity in the one case, by increased activity in the other. The possibility must, however, be also borne in mind that the *same* nerve fibres may set up both anabolic and katabolic changes, as when a secretory nerve is stimulated, provoking it may be for hours a discharge of products of katabolism from secretory cells; for it is in

such cases necessary to assume a continuous process of anabolism going on at the same time within the same cells.

Upon evidence founded mainly, but not exclusively, upon the investigation of certain electrical and visual phenomena, Hering has concluded that in all cases where either katabolic or anabolic changes are proceeding in any portions of bioplasm, they tend to render the bioplasm more and more resistant to the effects of the excitation which is producing the change (reaction); that in any given cell the longer or more strongly metabolic changes of the one character have been proceeding, the greater will be the tendency towards metabolic changes of the opposite character, so that even if, as may happen, in consequence of the action of an external stimulus (A), anabolic changes are proceeding at first more rapidly than katabolic, so that the balance is in favour of the building up or assimilation processes, the reaction which is thereby provoked will, after a time, by increasing the katabolism of the cell, tend again to produce a condition of balance. Only in this case the balance will be struck with the general bioplasm of the cell in a condition above par, as compared with that from which it was assumed to start (A—allonomous equilibrium). And, *mutatis mutandis*, increased katabolic processes due to external stimuli are (D) assumed to produce by reaction an increase of anabolism in adjacent portions of bioplasm, which increase becomes eventually sufficient to balance the increased katabolism induced by the stimulus, so that again the metabolism of the whole cell strikes a balance as it were, but now in a condition below par, as compared with the normal (D—allonomous equilibrium). Upon the cessation of the stimulus in either case, the tendency, say, to increased anabolism being removed with the stimulus, the opposite condition of increased katabolism, which was provoked by the increased anabolism, will for a time prevail, and there will be a falling off of the general assimilation of the cell, until what may be considered the normal condition is again established, the two processes again exactly balancing one another. And the same, *mutatis mutandis*, for the removal of a stimulus which was producing a condition of increased anabolism. There is thus assumed to be a sort of *internal self-adjustment of metabolism* in bioplasm.

It is a part of the theory of Hering that the anabolic and katabolic changes in the bioplasm are the direct or indirect cause of many, if not of all, physiological phenomena exhibited by living tissue, and that the prevalence of one kind of change in any portion of bioplasm will tend to start a change of the opposite kind in adjacent portions. But this is a subject which we need not here specially concern ourselves with, since the most important application of it to the explanation of physiological phenomena concerns the effects produced by the stimulation of the retina by light, and will be discussed in the article dealing with this question.

In connection with this subject, one other point must be borne in mind, namely, the possibility, indeed probability, that many metabolic changes in the body are not necessarily associated with the building up or breaking down of bioplasm, but are effected outside the actual molecules of which the bioplasm is composed, although under the influence of the activity of the bioplasm. Such changes as these may be distinguished from the metabolic changes of the bioplasm itself by the name of "contact changes," and they also involve both the building up of complex materials and the subsequent breaking down of such materials into simpler products associated frequently with oxidation. Such contact changes are analogous to those which are produced by organised ferments, such as yeast, outside the actual organism, although directly by its activity, and they must be sharply differentiated from the changes which the bioplasm itself is at the same time undergoing. This distinction will be referred to again in a subsequent section.

The understanding of the metabolic processes presupposes an acquaintance with the composition of the foodstuffs and of the bodystuffs, both of which

have been dealt with in previous articles. So far as the bodystuffs are concerned (and to a somewhat less extent with regard to the foodstuffs), it cannot be said that we possess an acquaintance so intimate as to enable us fully to understand the changes which they undergo; and as a consequence it will be found that our knowledge of metabolism, in spite of the enormous amount of work that has been done to elucidate it within the last five and twenty years, is still in an unsatisfactory condition.

Balance of nutrition.—The first determinations that require to be made in any inquiry into the metabolism of the body are those of its incomings and outgoings.[1] The incomings of the body consist of food and oxygen; the outgoings, of the various excreta, and of the carbon dioxide and water lost by the lungs and skin. If the incomings of the body exactly balance the outgoings, so that the animal neither gains nor loses weight, the body is said to be in *complete nutritive equilibrium.*

Sufficient information can be usually obtained regarding the balance of metabolism of the body, if the nitrogen and carbon only are determined in the ingesta and egesta.

As an instance of complete equilibrium in a man weighing 70 kilos., embracing both the nitrogen and carbon of the ingesta and egesta, the following balance table may be given (Burdon Sanderson[2]):—

Incomings.			Outgoings.		
Food.	N.	C.	Excreta.	N.	C.
Proteids . 100 grms.	15·5	53	Urine . . .	14·4	6·16
Fat . . 100 ,,	...	79	Fæces . . .	1·1	10·84
Carbohydrates 250 ,,	...	93	Respiration . .	...	208·00
	15·5	225		15·5	225

We may also have a condition in which the body either gains or loses weight, and in which consequently the incomings and outgoings do *not* exactly balance one another, but during which, nevertheless, the *nitrogen* which is taken into the body, and that which leaves the body, may strike an exact balance, while the other elements which compose the food and excreta, and especially the carbon, hydrogen, and oxygen, may not be similarly balanced. When the nitrogen of the food exactly balances the nitrogen excreted, the body is said to be in *nitrogenous equilibrium.* Under these circumstances we may assume that the living material of the tissues (which is essentially composed of nitrogenous substance) is neither diminished nor increased in amount; whereas, if at the same time the other constant elements of the food—the carbon, hydrogen, and oxygen—are met with in diminished or increased quantity in the excreta, we may assume that substances in the body other than the living tissues are either becoming laid on, or becoming diminished

[1] For the methods of determining these may be consulted, C. Voit in Hermann's "Handbuch," 1881, Bd. vi. S. 6 *et seq.*, and numerous papers which have appeared since then chiefly in the *Arch. f. d. ges. Physiol.*, Bonn (by Pflüger, Zuntz, and their pupils), and in the *Ztschr. f. Biol.*, München (by Voit and his pupils). See also v. Noorden, "Grundriss einer Methodik der Stoffwechsel-Untersuchungen," Berlin, 1892. For the methods of determining the respiratory products, see article "Chemistry of Respiration").

[2] "Syllabus of Lectures on Physiology," 1879.

in amount. These substances are mainly the fats, to a much less extent the carbohydrates, whereas the substances which form the actual tissues are composed of proteids and nucleo-proteids.

The following is an instance of a balance table[1] of a man weighing 70 kilos., showing nitrogenous equilibrium only, some of the carbon of the ingesta (mostly representing stored fat) not reappearing in the excreta :—

Incomings.			Outgoings.		
Foodstuffs.	N.	C.	Excreta.	N.	C.
Proteids . 137 grms.	19·5	315·5	Urine . . .	17·4	12·6
Fat . . 117 ,,	...		Fæces . . .	2·1	14·5
Carbo-hydrates 352 ,,	...		Respiration . .	...	248·6
	19·5	315·5		19·5	275·7

Whether the material which forms the bioplasm of the tissues has an essentially different molecular constitution during life from that which is met with in it after death, is not certainly known, but is extremely probable. This is obviously a point which is difficult of determination, because we cannot investigate the material composing bioplasm without previously killing it. All we are able to do is to determine, as far as possible, the changes which the tissues undergo, by investigating the products which they give off during life. Our knowledge of these products has led some physiologists to the conclusion that the substance of living material is composed of unstable cyanogen or aldehyde compounds, whereas it is well known that dead proteid yields bodies of an amide nature.[2]

Composition of foodstuffs.—The most important general fact that we need concern ourselves with in this place regarding the composition of foodstuffs is that, with ordinary mixed diet, they are composed in certain not very definite proportions of three chief kinds of organic material, namely, *proteids*, *carbohydrates*, and *fats*; in addition to which, *water* and *salts* are a necessary part of the food. The most general proportion of these three primary varieties of foodstuffs to one another in ordinary diet is found to be about one part of proteid material to from four to six parts of non-proteid, while the non-proteid constituents stand to one another in about the proportion of one part of fat to from five to ten parts of carbohydrate, this ratio having been arrived at by investigating the composition of freely chosen diets of persons in various occupations and stations of life. At the same time, it must be pointed out that departures from these proportions are by no means unfrequently met with, and especially is this the case with certain races of mankind, *e.g.* some of the Asiatic races, where a very much larger proportion of non-proteid material is ordinarily taken with the diet than is the case with Europeans; whereas, on the contrary, in parts of South America and Australia, where meat is plentiful, the proportion of proteid to non-proteid may be far larger than that above given.

[1] C. Voit, Hermann's "Handbuch," Bd. vi. S. 513. The table in the simplified form here given is from Neumeister, "Lehrbuch," Jena, 1897, Aufl. 2, S. 344.

[2] Cf. Halliburton, this Text-book, vol. i. p. 38.

On the whole, however, the above proportions are found to be fairly well maintained, the ratio of carbohydrates to fats in the diet varying more than the proportion of proteid to non-proteid material. As a general rule, it will be found that with the more wealthy classes there is a relatively greater amount of proteid and fat as compared with carbohydrates; whereas with the poorer classes the carbohydrates increase in proportion, and the proteids and fats diminish. With a diet composed of vegetable matter alone, the proportions are liable to be considerably modified, since, in order to obtain a sufficient amount of proteid from most vegetables, a much larger proportionate amount of carbohydrate food is inevitably consumed. On the other hand, since with flesh food the amount of proteid necessary for carrying on the metabolic processes of the body is much more easily obtained than from vegetable food, and since flesh food invariably contains a considerable amount of fat, the proportion of proteid and fat to carbohydrate is apt to be much greater than the normal when the diet is mainly composed of animal matter.

For the determination of the value of the chief organic materials of the foodstuffs in nutrition, the most important point to be ascertained regarding their composition is the amount of nitrogen and carbon in each. In round numbers, this may be stated as follows:—Proteids contain 15 to 17 per cent. N, and 50 to 55 per cent. C[1]; animal fats, on an average, 76·5 per cent. C; and carbohydrates, such as starch and sugar, 40 to 45 per cent. C. Since the amounts of proteid fat, and carbohydrates in all the ordinary foodstuffs has been accurately determined,[2] and is given in the form of tables, it is not difficult, if the amounts of each which are ingested are carefully weighed, to determine by calculation the total N and C of the ingesta. For very accurate work, however, it is necessary to make direct determinations of the N and C in the food taken; this is effected by ordinary chemical methods (that of the nitrogen usually by Kjeldahl's method).

The amount of flesh or fat which is at any time becoming lost or laid on can be easily approximately determined by an examination of a balance table, for the nitrogen in the urine represents metabolised proteid, the amount of which is arrived at by multiplying the numbers of grms. of nitrogen found by 6·25 (since proteids contain 16 per cent. N). Since any excess or deficit of proteids represents flesh lost or laid on, the amount of such loss or addition can be directly obtained by taking each gramme N in excess or deficit to represent 30 grms. flesh (since flesh contains about 3·4 per cent. N) (Voit). And, after reckoning off the carbon which the proteid metabolised would contain (53 per cent.), any further excess or deficit of carbon in the ingesta would represent the carbon of fat lost or laid on, and the amount of this may be approximately obtained by multiplying the number of grms. of carbon in the excess or deficit by 1·3 (since fat contains about 76·5 per cent. carbon). Thus, in the balance table on p. 872, the man under observation retained 39·8 grms. C, representing 52 grms. fat laid on.

The following table (from Bunge) gives the percentage composition of some of the chief foodstuffs; the remainder in each case is mainly water with a variable amount of salts—the numbers are taken from König. They are given in inverse order to the proportion of proteid they contain:—

[1] Argutinsky determined the percentage composition of beef, completely divested of fat and dried, to be as follows:—C 49·6, N 15·3, H 6·9, O+S 23·0, ash 5·2 (*Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. lv. S. 345).

[2] König, "Chemie der menschl. Nahrungs-u. Genussmittel," Berlin, 1882, Aufl. 2.

Foodstuff.	Proteid.	Fat.	Carbohydrate.
Apples . . .	0·4	...	13
Carrots . . .	1·1	0·2	9
Potatoes . . .	2	0·1	20
Human milk . .	2	4	6
Cabbages . . .	3·3	0·7	7
Cow's milk . .	3·4	4	5
Rice	8	0·9	77
Maize . . .	10	4·6	71
Wheat . . .	12	1·7	70
White of eggs . .	13	0·3	...
Fat pork . . .	15	37	...
Yolk of eggs . .	16	32	...
Fat beef . . .	17	26	...
Fish (pike) . .	18	0·5	...
Lean beef . . .	21	1·5	...
Peas	23	1·8	58

Heat value of foodstuffs.—A most important determination to be made regarding any diet is its caloric (calorific) value. This is arrived at by multiplying the number of grammes of its several organic constituents by a number, determined by exact experiment, representing the amount of heat produced by the oxidation of 1 grm. of the carbohydrate, fat, or proteid to water and carbon dioxide and to urea. Such calorimetric experiments were first carried out systematically by Frankland,[1] who determined the caloric value of various articles of diet, and his results have since been extended and confirmed or amended by various observers,[2] using improved calorimetric methods.

According to Rubner, the average caloric value of the proteid of the aliment is 4124 calories, *i.e.* 1 grm. proteid oxidised to urea yields 4124 grm. degrees (or 4·1 kilogram-degrees) of heat; of the fat, 9321 calories (9·3 kilogram-degrees); and of the carbohydrate (starch), 4116 calories (4·1 kilogram-degrees). Applying these numbers to Voit's diet (see next page), we obtain in round numbers—

105	grms.	assimilated proteid	× 4·1 =	430
56	,,	fat	× 9·3 =	520
500	,,	carbohydrate	× 4·1 =	2050
			=	3000 kilo-calories,

or 3,000,000 calories, as the energy value per diem of the food of a man of about 70 kilos., doing hard muscular work.

This amount is probably a little too high, since the whole of the fat and carbohydrate of a mixed diet is not assimilated. Rubner estimates the actual production at 2,843,000 calories. Hultgren and Lantergren, however, found that Swedish workmen, of an average weight of only 67 kilos., consumed on an average per diem 159 grms. proteid, 93 grms. fat, and 570 grms. carbohydrate, which, even allowing for the non-assimilation of a certain proportion of each, would still give a higher caloric value for the total foodstuffs. Probably,

[1] "On the Origin of Muscular Power," *London, Edinburgh, and Dublin Phil. Mag.*, London, 1866, vol. xxxii. p. 182.

[2] Stohmann, *Journ. f. prakt. Chem.*, Leipzig, 1879, Bd. xix. S. 115; *Ztschr. f. Biol.*, München, 1894, Bd. xxxi. S. 364; Danilewsky, *Arch. f. d. ges. Physiol.*, Bonn, 1885, Bd. xxxvi. S. 237; Rubner, *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 313; 1885, Bd. xxi. S. 250 u. 337; 1886, Bd. xxii. S. 40; 1894, Bd. xxx. S. 73.

therefore, 3000 kilo-calories may be taken as a fair average for the caloric value of the ingesta of a man weighing about 70 kilos., which would give about 43 calories for each kilogram body weight.[1]

In women the amount is somewhat less than this, both absolutely and also relatively. In children, though absolutely less, it is relatively greater.

Since the combustion of 1 grm. fat produces 9·3 kilo-calories, and the combustion of 1 grm. proteid to urea CO_2 and H_2O and of 1 grm. starch to CO_2 each produces 4·1 kilo-calories, the combustion of 100 grms. fat will produce an equal amount of energy with the combustion of 227 grms., either of proteid or of starch. This amount, therefore, of proteid or of starch is said to be of the same "isodynamic value" as 100 grms. fat. It has been shown by the carefully conducted calorimetric investigations of Rubner,[2] that the isodynamic values are as nearly as possible the same, whether the combustion occurs in air, or in the tissues of the animal body.[3]

Minimal amount of proteid necessary in food.—There has been much disputation as to the minimal amount of proteid which it is possible for a man in health and doing work to take in his diet in the course of twenty-four hours. Ranke gave as a normal diet for an average man (70 kilos.) not engaged in muscular work, 100 grms. of proteid, 100 grms. of fat, and 240 grms. of carbohydrate.[4] Voit allowed for a man of 70 to 75 kilos., doing ten hours' muscular work, 118 grms. of proteid, 56 grms. of fat, and 500 grms. of carbohydrate.[5] It has, however, been shown that, provided the non-proteids in the diet are increased—not only in proportion to the caloric value of proteid withdrawn, but considerably more than in such proportion—a man can maintain equilibrium and can do work upon considerably less proteid than that allowed in the diets of Ranke and of Voit.

Thus Hirschfeld (70 kilos.)[6] found that he could maintain himself for a considerable time in perfect health with a diminution of proteid down to 75 grms., or even for a time down to 49 grms. per diem, but under these circumstances it was necessary to increase enormously the amount of non-proteid and especially of carbohydrate material taken with the diet.

[1] Hultgren and Lantergren, working with Tigerstedt, found the heat value of the food of six persons living on a freely chosen diet to vary from 33 to 49 calories per kilogramme. They found that the heat value of the proteid was about 16 to 19 per cent. of the total heat value of the food, that of the fat being about 21 to 24 per cent., and that of the carbohydrate about 60 per cent. Ranke's diet (*vide infra*), with a heat value of only 2,365,000 calories, is for a man performing no muscular work.

[2] *Ztschr. f. Biol.*, München, 1894, Bd. xxx. S. 73.

[3] A more complete account of the heat values of the foodstuffs is given in the article on "Animal Heat" in p. 833. For the influence of food on the respiratory exchange, see article "Chemistry of Respiration," p. 717; see also Magnus-Levy, *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. lv. S. 1.

[4] To this may be added 25 grms. salts and 2535 grms. water (including that contained in the solid food). These several constituents are contained in a daily ration of 250 grms. meat, 400 grms. bread, 70 grms. starch or sugar, 100 grms. fat, 10 grms. salt, and 2100 water (J. Ranke, "Die Ernährung des Menschen," München, 1876).

[5] Such a diet contains about 18·3 grms. N, and about 328 grms. C, whereas Ranke's diet contains about 15·5 grms. N, and about 220 grms. C. It should be added that about 13 grms. of the 118 grms. proteid of Voit's diet is not absorbed or assimilated, so that the available proteid is about 105 grms. This closely corresponds with the results of Bleibtreu and Bohland (with Pflüger), who give 1·5 grms. per kilo. body-weight. This would be a little over 105 grms. for a man weighing 70 kilos. (*Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxviii. S. 1). In Hultgren and Lantergren's observations the actual amount of the ingested proteid which underwent metabolism averaged 101·3 grms.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1887, Bd. xli. S. 533; and 1889, Bd. xliv. S. 428; also *Virchow's Archiv*, 1888, Bd. cxiv. S. 301.

Klemperer[1] reduced the amount of proteid in his own diet to as little as 25 grms. per diem, but required 262 grms. fat, and 406 grms. carbohydrate (with a total caloric value of more than 5,000,000 calories) to maintain equilibrium.

I. Munk brought a dog into nitrogenous equilibrium with a diet consisting mainly of proteid. If, now, one-half the proteid of the diet was removed and replaced by non-proteid, an amount of non-proteid having a caloric value of about two-fifths more than that of the proteid removed was required to maintain equilibrium; and the more the proteid removed from the diet, the greater the proportionate amount of non-proteid required. Ultimately, the amount of proteid was reduced to 1·5 grms. per kilo. body-weight; under these circumstances an amount of non-proteid, twelve to fifteen times the caloric value of the proteid removed, was required to maintain equilibrium.[2] After the lapse of some weeks, the animal failed properly to digest the large amount of non-proteid required, and it became necessary to reduce this and increase the proteid.

The amount of nitrogen taken in these experiments was distinctly less than the amount which would be lost in the fasting condition.

Of the two chief kinds of non-proteid food, v. Noorden and Kayser[3] have found that carbohydrates are of greater value as proteid-sparers than fats. In a mixed diet, therefore, containing just enough proteid and non-proteid for the needs of the economy, fats cannot be substituted for their caloric equivalent of carbohydrates without loss of proteid occurring. Gelatin is of still greater value as a proteid-sparing food than are either fats or carbohydrates (see p. 878), and by its use, although it cannot be built up into tissue, the amount of tissue proteid lost from the body can be reduced, according to Voit, to about the half of that which is normally lost, and which on Voit's estimate amounts to about 33 grms. daily,[4] or 1 per cent. of the actual living substance.[5] The importance of gelatin as an article of diet will be specially treated of later on.[6]

In spite of such experiments, it may be doubted whether a diet which includes considerably less proteid than 100 grms. for the twenty-four hours could maintain a man of average size and weight for an indefinite time. It has frequently been asserted that many Asiatics consume a very much smaller proportion of proteid than is the case with Europeans. The inhabitants of India, Japan, and China chiefly consume rice as the normal constitution of their diet, which contains relatively little proteid; and this has been advanced as an argument in favour of the view that the minimal amount of proteid is much less than that ordinarily given as essential to the maintenance of nutritive equilibrium. It must, however, be stated that we have no definite statistics to show that, in

[1] *Arch. f. Physiol.*, Leipzig, 1889, S. 361. Similar experiments have been made by Peschel (Diss., Berlin, 1890) and Graham Lusk, *Ztschr. f. Biol.*, München, 1891, Bd. xxvii. S. 459. See also E. Voit, *München. med. Wchnschr.*, 1889, S. 748; and C. Voit, *ibid.*, 1891, S. 195.

[2] *Arch. f. Physiol.*, Leipzig, 1891, S. 338 (*Verhandl. d. physiol. Gesellsch.*) and *Virchow's Archiv*, 1893, Bd. cxxxii. S. 91. See also Rosenheim, *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 61; and Ritter, *München. med. Wchnschr.*, 1893, Nos. 31 and 32.

[3] *Arch. f. Physiol.*, Leipzig, 1893, S. 371.

[4] The half of this amount, since it can be replaced by gelatin, is set down by Voit to disintegration of "circulating proteid" instead of actual "tissue proteid."

[5] Hermann's "Handbuch," Bd. vi. S. 302, and *Ztschr. f. Biol.*, München, 1889, Bd. vii. S. 284.

[6] Pagliese, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1897, S. 329, has found that fats, carbohydrates, and gelatin, not only diminish the amount of the nitrogen excreted, but also the phosphoric acid, and this even in a greater proportion, and probably by diminishing the waste of the nucleo-proteids of the tissues.

proportion to their body weight, Asiatics doing the same amount of work as Europeans require a less amount of proteids; indeed, such evidence as is forthcoming is rather in favour of the opposite conclusion.[1]

The following table (from Hultgren and Lantergren) gives the average amounts of the proteids, fats, and carbohydrates in freely chosen diets of workmen of different countries, together with the total heat values of such diets:

		Proteid.	Fat.	Carbohydrate.	Kilocalories.
Moderately hard work	Russian workmen (Erisman) .	131·8	79·7	583·8	3675·2
	Munich workmen (Forster) .	131·9	81·5	457·4	3174·1
	Swedish workmen (H. and L.) .	134·4	79·4	522·8	3436
Hard work	Swedish workmen (H. and L.) .	189	110	714	4726

With these may be compared the following:—

	Proteid.	Fat.	Carbohydrate.	Kilocalories.
Soldiers on active service (Voit) .	145	100	500	3574·5

The average proportion of proteid to non-nitrogenous constituents of the food is given by Hultgren and Lantergren at 1 : 4·27 by weight, and 1 : 4·95 by heat value; of fat to carbohydrate at 1 : 6·34 by weight, and 1 : 2·80 by heat value.

The manner in which the proteid and non-proteid constituents of the diet are most advantageously taken into the body, or, in other words, the constitution of dietaries, forms a subject belonging more properly to the domain of personal hygiene. It would, moreover, occupy far too much space to discuss at all adequately the constitution of diets of different people and in different countries. It is sufficient to state that under ordinary circumstances the proteids are taken in such forms as flesh, egg, and cheese, bread and other cereals, and leguminous foods, the fat in the form of meat-fat and butter, and the carbohydrate in the form of starch or cane-sugar derived from or contained in vegetable food. With a purely vegetarian diet the proteid of the food may be derived largely from the leguminous plants and to a somewhat less extent from the cereals, and the fat from the seeds of plants.[2] We may now proceed to consider the effects upon nutrition of some of the more important constituents of the diet.

[1] Cf. Kumagawa, *Virchow's Archiv*, 1889, Bd. cxvi. S. 370; Kellner and Mori, *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 102; I. Munk, *ibid.*, 1893, Bd. cxxxii. S. 91.

[2] For statistics concerning diet see J. Ranke, "Die Ernährung des Menschen," München, 1876; C. Voit in Hermann's "Handbuch," Bd. vi. ("Physiologie des allgemeinen Stoffwechsels und der Ernährung"), Leipzig, 1881; König, "Chem. d. menschl. Nahrungs-u. Genussmittel," Berlin, 1882, Aufl. 2; I. Munk and Uffelmann, "Ernährung des Menschen," Wien u. Leipzig, 1887, in which also the literature of the subject up to that date will be found; Scheube, *Mitth. d. deutsch. Gesellsch. f. Nat.-u. Völkerk. Ostasiens*, Yokohama, 1882, No. 24, and *Arch. f. Hyg.*, München u. Leipzig, 1884, Bd. i. S. 352 (diet of Japanese); Hultgren and Lantergren, "Untersuch. ü. d. Ernähr. Schwedischer Arbeitern," Stockholm, 1891; Studemund, *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlviii. S. 578; Ohlmüller, *Ztschr. f. Biol.*, München. 1884, Bd. xviii. S. 393; G. Bunge, "Der Vegetarianismus," Berlin, 1885; Kumagawa, *Virchow's Archiv*, 1889, Bd. cxvi. S. 370; Albertoni and Novi, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lvi. S. 213 (criticised by Hultgren, *ibid.*, 1895, Bd. lx. S. 205). Diet statistics will also be found in most text-books of physiology.

Special constituents of the diet.—Proteids.—Proteids are chiefly taken in the diet in the form of egg-albumin, vitellin, myosin, casein, the proteids of cereals and of leguminous seeds (mainly globulins). The nutritive value of the proteids from any one of these sources is pretty nearly the same, with the exception that somewhat less of the proteid of vegetable food is digested and assimilated than that of animal origin, and the less, the larger the amount of cellulose which is contained in the food.

Peptones and albumoses have about the same caloric and nutritive value as the proteids from which they have been formed.[1] Certain proteids are assimilated and have the same nutritive value, if injected into the blood vessels or under the skin, as when digested and absorbed from the intestines. This is the case with serum-albumin and serum-globulin, and also with acid or alkali albumin (even if prepared from egg-albumin) and phytovitellin.[2] Other forms of proteid are not thus directly assimilable, but on injection appear at once in the urine. Such are egg-albumin,[3] casein,[4] peptone, and albumoses. Hæmoglobin must also be reckoned with these, although if injected as blood (with the blood corpuscles intact), it remains intact. If injected dissolved in water or in serum, it becomes partly broken up and converted into bile pigment and partly appears in the urine as hæmoglobin.

Most, if not all, proteids contain sulphur, and the nucleo-proteids contain phosphorus; an increase of sulphates and sometimes of phosphates in the urine may therefore be expected, if their metabolism is increased. The metabolism of proteids will be subsequently dealt with.

Gelatin.—Gelatin, although its elementary composition is very nearly the same as that of proteids, and although it becomes, like proteids, converted into peptones by digestion, and after being assimilated is oxidised into urea CO_2 and H_2O, is different from proteids in its chemical constitution (see "Chemical Constituents of Body and Food, pp. 31 and 70), and cannot wholly replace proteid as an article of diet. This arises from the fact that the bioplasm of the tissues is unable to be produced from it. In spite, therefore, of its containing nitrogen and all the elements of the proteid molecule, it is a non-proteid food, and takes its place as such along with the fats and carbohydrates. Like them also it acts as a proteid-sparer, so that a certain amount of proteid can be removed from the diet and replaced by gelatin; about twice as much of this must, however, be added, as proteid is removed.[5] As a proteid-sparer, gelatin acts more efficiently than carbohydrates, and still more than fats. This is shown by an experiment by Voit upon a dog weighing 32 kilos., which had been maintained very nearly on nitrogenous equilibrium by a daily allowance

[1] Politzer, *Arch. f. d. ges. Physiol.*, Bonn, 1885, Bd. xxxvii. S. 301.

[2] Plósz and Gyergai, *Arch. f. d. ges. Physiol.*, Bonn, Bd. ix. S. 325, and Bd. x. S. 536; Maly, *ibid.*, Bd. ix. S. 605; Adamkiewicz, *Virchow's Archiv*, Bd. lxxv. S. 144; Stokvis, *Centralbl. f. d. med. Wissensch.*, Berlin, 1864, S. 596; Lehmann, *Virchow's Archiv*, 1864, Bd. xxx. S. 593; Ponfick, *Virchow's Archiv*, 1875, Bd. lxii. S. 273; Forster, *Ztschr. f. Biol.*, München, 1875, Bd. xi. S. 517; Tizzoni, *Arch. ital. de biol.*, Turin, 1884, Bd. vi. S. 395; Neumeister, *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1889, S. 64; *Ztschr. f. Biol.*, München, 1891, Bd. xxvii. S. 309.

[3] Bernard, "Leçons sur les propr. physiol. etc.," Paris, 1859, tome ii. p. 467.

[4] Runeberg, *Deutsches Arch. f. klin. Med.*, Leipzig, 1879, Bd. xxiii. S. 68.

[5] According to Voit, one-fifth of the ordinary amount of proteid may be so replaced. I. Munk, however, in the dog got at least two-thirds of the proteid of the food replaced by gelatin with maintenance of equilibrium (*Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 309, and Bd. lxi. S. 607).

of 500 grms. of meat. On removing 100 grms. of this from the diet, and replacing it by 200 grms. of gelatin, there was a gain of nitrogen to the body representing the putting on of 44 grms. of flesh, whereas when the 100 grms. of meat was replaced by 200 grms. of fat, or by 250 grms. of starch, there was a loss of nitrogen representing a loss of flesh to the amount respectively of 50 and 39 grms.

The following experiments of Voit on a dog are also instructive. The numbers represent grammes:—

Expt.	Lean Meat.	Gelatin.	Flesh lost or gained.
1.	500	0	−22
	500	200	+54
2.	2000	0	+30
	2000	200	+376
3.	200	200	−118
	200	300	−82
4.	200	200	+25
	0	200	−118

That it cannot wholly replace proteid is shown by the fact that even when very large quantities are given either alone or in combination with fat and carbohydrate, an excess of nitrogen appears in the excreta —in other words, there is still a loss of flesh from the body.[1] To a certain extent gelatin will act as a fat-sparer, *i.e.*, when given along with proteid, it may prevent the oxidation of body fat, but its activity in this respect is far below that of either fats or carbohydrates.[2] Even the collagenous tissues can apparently not be formed from gelatin ingested, since this wholly appears (as urea, etc.) in the excreta; these tissues must therefore be formed, like all others, from proteid food.[3] Gelatin is also not assimilated if injected into the blood or under the skin; it appears at once in the urine.[4]

Nucleins and **nucleo-proteids**, as well as **lecithins**, are found in all forms of mixed diet; and although nuclein is not digested by the gastric juice, nor, according to Bokay,[5] by artificial pancreatic juice, there are reasons for believing that a part at least of the nuclein of the food is absorbed and converted in the body into other substances. It is found, for example, that the ingestion of foodstuffs containing much nuclein causes a marked increase of uric acid in the urine,[6] and, as we shall show later on, there is strong reason to believe that the iron necessary for the formation of hæmoglobin is derived from some forms of nuclein.

[1] For the evidence of this, see C. Voit, *op. cit.*, S. 122.

[2] C. Voit, *op. cit.*, S. 126.

[3] An interesting historical account of the question of gelatin as an article of diet is given by Voit (*op. cit.*, S. 395).

[4] Cl. Bernard and Barreswil, *Journ. de pharm. et chim.*, Paris, 1844, Sér. 3, tome v. p. 425.

[5] *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 157.

[6] Horbaczewski, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1891, Bd. c. Abth. 3, S. 78.

The phosphorus of any nuclein which is absorbed is probably converted into phosphoric acid, and excreted as phosphates by the urine. There is no evidence that the nuclein which is absorbed is taken up by the tissues, and by them again converted into tissue nucleins; it is more probable that these arise by independent synthesis from proteid and phosphates. That this may occur was shown by Miescher,[1] who found in the case of the salmon, which travels from the sea to the upper Rhine, there to deposit its spawn, and which during the whole period of its journey and sojourn in the river, lasting some weeks or even months, takes no food whatever (the alimentary canal being always found empty), that the ovaries increase in size at the expense of the muscular tissue. Now the ovaries, being mainly composed of ova, contain large quantities of nuclein and lecithin, whereas the muscles contain mainly ordinary proteids and very little of these substances; the latter must therefore be formed by synthesis, the materials for such synthesis being derived from the proteids, the fats, and the phosphates of the muscles.

Amido-acids. — Experiments to determine the nutrition, and especially the proteid-sparing value of amido-acids, have chiefly been made with asparagin, which occurs in some quantity in certain vegetables. The general result of these inquiries is to show that in herbivora (rabbit, goose, sheep), the amido-acids can act as proteid-sparers, whereas in carnivora (dog) and omnivora (rat) they have not proteid-sparing effects when added to the diet.[2]

Creatine has been found to have no nutritive value. If given with the food, it appears wholly in the urine as creatinine.[3]

Carbohydrates.—Apart from the small amount of glycogen or sugar which may be contained in flesh foods, and from the lactose of milk, the carbohydrates of the food are wholly derived from the vegetable kingdom. The chief carbohydrate constituents of an ordinary diet are starch and cane-sugar, with a certain amount of grape-sugar when there is much consumption of certain fruits. Neither starch (in solution) nor cane-sugar (Bernard) is directly assimilable when injected into the blood vessels, and the same is true for maltose and lactose.[4] These substances all appear under such circumstances at once in the urine.

On the other hand, dextrose can be directly assimilated, even in large amounts. It is necessary that the injection should be conducted slowly, so that the liver should have time to convert it into glycogen before the proportion of dextrose in the blood much exceeds about 0·2 per cent. Injected too rapidly, or in too large doses (more than 1 grm. per kilo. body weight), glycosuria results;[5] and if its elimination by the kidneys

[1] *Arch. f. Anat. u. Entwcklngsgecch.*, Leipzig, 1881, S. 193; and "Statistische u. biol. Beitr. z. Kenntniss vom Leben des Rheinlachses," 1880 (quoted from Bunge's "Handbuch").

[2] Weiske, *Ztschr. f. Biol.*, München, 1879, Bd. xv. S. 261; 1881, Bd. xvii. S. 415; 1884, Bd. xx. S. 277; 1894, Bd. xxx. S. 254; Zuntz and Bahlmann, *Arch. f. Physiol.*, Leipzig, 1882, S. 424 (*Verhandl. d. phys. Gesellsch.*); Potthast, *Arch. f. d. ges. Physiol.*, Bonn, 1883, Bd. xxxii. S. 280; I. Munk, *Virchow's Archiv*, 1883, Bd. xciv. S. 436; and 1884, Bd. xcviii. S. 364; Mauthner, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 507; E. Voit, *Sitzungsb. d. k.-bayer. Akad. d. Wissensch. zu München*, 1883, S. 401; *Ztschr. f. Biol.*, München, 1892, Bd. xxvii. S. 492; 1893, Bd. xxix. S. 125; Gabriel, *ibid.*, S. 115.

[3] Meissner, *Ztschr. f. rat. Med.*, 1868, Bd. xxxi. S. 283.

[4] According to Dastre (*Arch. de physiol. norm. et path.*, Paris, 1889, p. 718), *galactose* is directly assimilable.

[5] Biedl and Kraus (*Wien. klin. Wchnschr.*, 1896, S. 55) state, however, that they were able to inject as much as 200 to 300 grms. of grape-sugar, in 10 per cent. solution, into the vein of a man, without producing either polyuria nor any but a slight temporary glycosuria.

be prevented, as by tying the ureters, the excess of sugar undergoes changes which result in the formation of lactic acid, acetone, diacetic acid, and other substances, the production of which is accompanied by convulsions, and eventually coma, as in severe natural diabetes.[1]

Very large amounts of starch can be taken into the alimentary canal, and corresponding amounts of dextrose absorbed into the blood, without producing glycosuria in a normal animal. But if the assimilation powers have been reduced by starvation, glycosuria is found to occur on the ingestion of a large amount of starch.[2] On the other hand, if cane-sugar, maltose or lactose, and even lævulose, are taken by the mouth in large quantities, even without a previous starvation period, part of the sugar ingested appears in the urine (alimentary glycosuria).[3] This is apparently due to the fact that the blood vessels of the intestine cannot carry away all the absorbed sugar with sufficient rapidity to the liver, and some of it consequently passes to the general circulation by way of the thoracic duct,[4] and thus to the kidneys, which always immediately eliminate any excess of sugar in the blood passing through them. Glycosuria also occurs when sugar solutions are injected into the large intestine of dogs.[5]

Cellulose is not readily digested by carnivora nor by man, but in some forms of food (carrots, cabbage, celery, lettuce) a considerable proportion of the cellulose present may become dissolved and absorbed ;[6] in herbivora it undergoes digestion, and is eventually absorbed as dextrose. Its chief value in the diet of animals seems, however, to be due to its action in promoting peristalsis of the intestines. Rabbits die from inflammation of the intestines if devoid of cellulose ; its place can be supplied in them by horn-shavings, which have the same mechanical effect. In carnivora and man this is not so important, as the gut is shorter, but probably the cellulose of mixed food tends to prevent constipation. A purely milk diet is well known to be constipating (Bunge).

The fate of the carbohydrates after assimilation will be treated of in a special section on carbohydrate metabolism.

Fats are taken in largely in the form of animal fat (fats of flesh and milk), but also largely, especially in some countries, in the form of vegetable fats, such as olive oil and the fats met with in certain seeds. In the last-named form they are protected by cellulose, and are far less easily digested and assimilated. The changes which they undergo in the processes of digestion and absorption have already been fully considered (pp. 443–463), also their caloric value, and their importance as proteid-sparers. Their assimilation to the natural fat of the body, and their formation within the body, will be treated of subsequently.

Fatty acids and soaps have been shown by I. Munk (in dogs) to have very nearly the same nutritive value as the fats from which they

[1] V. Harley, *Arch. f. Physiol.*, Leipzig, 1893, Suppl., S. 46.

[2] Hofmeister, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxv. S. 240 ; and 1890, Bd. xxvi. S. 355.

[3] Worm-Müller, *Arch. f. d. ges. Physiol.*, Bonn, 1884, Bd. xxxiv. S. 576 ; Hofmeister, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxv. S. 240 ; C. Voit, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 265 ; Miura, *ibid.*, 1896, Bd. xxxii. S. 281.

[4] Ginsberg, *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xliv. S. 306.

[5] Eichhorst, *ibid.*, 1871, Bd. iv. S. 601.

[6] Weiske, *Ztschr. f. Biol.*, München, 1870, Bd. vi. S. 456 ; Knierem, *ibid.*, 1885, Bd. xxi. S. 67. See also Luntz, *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlix. S. 477.

are formed. This has, however, been already discussed (p. 750), and will be again referred to later on.

Glycerin has been found to act in some measure as a fat and carbohydrate-sparer, but not as a proteid-sparer.[1] Of the total amount ingested, from 21 to 37 per cent. is secreted in the urine unaltered, when given in large doses.[2] The sparing effect of glycerin on the conversion of liver glycogen into sugar will be subsequently referred to.

Alcohol.—The nutritive value of alcohol has been the subject of considerable discussion, and not a few experiments. Some of these tend to show that in moderate non-poisonous doses it acts as a non-proteid food in diminishing the oxidation of proteid, doubtless by becoming itself oxidised.[3] Its action, however, in this respect is relatively small, and indeed a certain proportion of alcohol ingested is exhaled with the air of respiration. Moreover, in large doses, it may act in the contrary manner, increasing the waste of tissue proteid.[4] It cannot, in fact, be doubted that any small production of energy resulting from its oxidation is more than counterbalanced by its deleterious influences as a drug upon the tissue elements, and especially upon those of the nervous system.

It is of interest, in connection with this subject, to point out that alcohol has been regarded by some physiologists as probably formed at a stage in the metabolism of carbohydrates prior to their complete oxidation, traces of alcohol having been obtained from fresh tissues by distillation with water.[5]

Inorganic substances.—Mineral salts, especially chloride of sodium and phosphates of lime and of the alkalies, are essential parts of any diet. The following table from Bunge gives the proportions

	K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	P_2O_5.	Cl.
Beef	1·66	0·32	0·029	0·15	0·02	1·83	0·28
Wheat	0·62	0·06	0·065	0·24	0·026	0·94	?
Potato	2·28	0·11	0·100	0·19	0·042	0·64	0·13
White of egg . . .	1·44	1·45	0·130	0·13	0·026	0·20	1·32
Peas	1·13	0·03	0·137	0·22	0·024	0·99	?
Human milk . . .	0·58	0·17	0·243	0·05	0·003	0·35	0·32
Yolk of egg . . .	0·27	0·17	0·380	0·06	0·040	1·90	0·35
Cow's milk . . .	1·67	1·05	1·51	0·20	0·003	1·86	1·60

[1] I. Munk, *Virchow's Archiv*, Bd. lxxvi. S. 119 ; Bd. lxxx. S. 39.

[2] Tschirwinsky, *Ztschr. f. Biol.*, München, 1880, Bd. xv. ; Arnschink, *ibid.*, 1888, Bd. xxiii. S. 413.

[3] Strassmann, *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. xlix. S. 315. Chittenden (*Journ. Physiol.*, Cambridge and London, 1892, vol. xii. p. 220), experimenting upon dogs, obtained very little influence on proteid metabolism. For the earlier literature of this question, cf. C. Voit, *op. cit.*, pp. 169 and 415.

[4] Miura, *Ztschr. f. klin. Med.*, Berlin, 1892, Bd. xx. S. 137. I. Munk obtained similar results upon dogs (*Verhandl. d. Physiol. Gesellsch.*, 1878–79, No. 6 in *Arch. f. Physiol.*).

[5] Hoppe-Seyler and Rajewsky, *Arch. f. d. ges. Physiol.*, Bonn, 1875, Bd. xi. S. 122.

per cent. in which the different salts of the ash occur in *dried* food-stuffs.[1]

Animals from whose food the salts have been extracted, sometimes die even more rapidly than animals which have been altogether deprived of food, with the supervention of various symptoms indicating a disturbance of the central nervous system and of the digestive system.[2] This more rapid end of such animals is due, according to Bunge,[3] to chronic acid-poisoning, produced by the oxidation of the sulphur of the proteids; such acid being normally neutralised by the basic salts (phosphates, carbonates, and alkali-albuminates) taken in with the food, whereas in the absence of these, basic substances are removed from the tissues to take their place. The experiments of Lunin (in Bunge's laboratory) upon mice fed respectively upon salt-free food, or upon the same food to which sufficient sodium carbonate was added to exactly neutralise the sulphuric acid which would be formed in the oxidation of the proteid of the food, seem to show that Bunge's conjecture is correct; for such animals lived considerably longer than those to which no soda was given, or than those to which it was given combined with chlorine.[4] This, however, is probably not the whole explanation, for in both the dog and man the faculty of resisting the effects of acids in the ingesta depends in part, at least, on their neutralisation by ammonia, which is derived from metabolised proteid.[5]

It would appear that some at least of the mineral matters of the food must be in their natural condition, which is probably that of combination with the proteid substances. For Lunin found that although mice will live indefinitely on desiccated milk, yet if they are given an artificial food consisting of a mixture of salt-freed casein and lactose, to which have been added the same inorganic salts which are present in the original milk, the animals will die at about the same period as if sodium carbonate alone had been added to the casein and sugar.[6]

As Bunge has pointed out, the addition of **chloride of sodium** to the ordinary food appears to be essential to the well-being of all animals the food of which contains a large proportion of potassium salts, as occurs in most vegetables. In conformity with this, we find that those races of mankind which subsist mainly on vegetable food find salt an absolute necessity of life; and that the same is the case with herbivorous animals is shown by the fact that these are often found to travel hundreds of miles to reach a place where salt is to be found (salt-licks). Carnivorous animals, on the other hand, and those herbivora which consume plants and herbage which do not contain a great excess of potassium salts, show no such inclination to seek salt. The same is true for those races of mankind who live almost exclusively on fish or flesh,

[1] Note especially the small amount of Na_2O in wheat and peas; the large amount of CaO in milk and egg yolk, and the very small amount of iron in milk. On the other hand, the ash of the fœtus contains a very large proportionate amount of iron.

[2] Forster, *Ztschr. f. Biol.*, München, 1873, Bd. ix. S. 297.

[3] *Ztschr. f. Biol.*, München, 1874, Bd. x. S. 130. See also "Lectures," pp. 114–118.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1881, Bd. v. S. 31. See also Socin, *ibid.*, 1891, Bd. xv. S. 100.

[5] Schmiedeberg and Walter, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, Bd. vii. S. 148; Hallervorden and Coranda, *ibid.*, Bd. xii. S. 76.

[6] Somewhat similar conclusions were arrived at by Bunge and Socin from experiments upon another artificial food, which had been first deprived of salts, but to which these were afterwards added. This food, although apparently containing all needful materials for nutrition, was unable to keep the mice which were fed upon it alive.

or on such vegetable food, *e.g.* rice, in which the potassium salts are only present in small quantity. It is further noteworthy that the peoples who live on an animal diet, without salt, carefully avoid a loss of blood when they slaughter the animals, for the blood contains a far larger amount of sodium in proportion to potassium than any other tissue or organ. The explanation of these facts is thus offered by Bunge[1]:—

"The amount of salt which herbivorous animals take in with their food is, compared with the weight of the body, generally not much less than that consumed by carnivorous animals. On the other hand, there is a considerable difference in another constituent of the ash of their food, in the potassium. Herbivorous animals take at least three or four times as much of salts of potassium as the carnivora. This fact leads me to imagine that the abundance of potassium in vegetable food is the cause of the need for salt in the herbivora. If, for instance, a salt of potassium, such as potassium carbonate, meets with common salt or chloride of sodium in solution, a partial exchange takes place—chloride of potassium and carbonate of sodium are formed. Now, chloride of sodium is well known to be the chief constituent among the inorganic salts of blood plasma. When, therefore, salts of potassium reach the blood by the absorption of food, an exchange takes place. Chloride of potassium and the sodium salt of the acid which was combined with the potassium, are formed. Instead of the chloride of sodium, therefore, the blood now contains another sodium salt, which did not form part of the normal composition of the blood, or at any rate not in so large a proportion. But the kidneys possess the function of maintaining the same composition of the blood, and of thus eliminating every abnormal constituent, and any excess of a normal constituent. The sodium salt formed is therefore ejected by the kidneys, together with the chloride of potassium, and the blood becomes poorer in chlorine and sodium. Common salt is therefore withdrawn from the organism by the ingestion of potassium salts. This loss can only be made up from without, and this explains the fact that animals which live on a diet rich in potassium have a longing for salt."

In confirmation of this deduction, Bunge found that the addition of potassium salts to his diet produced a striking increase in the excretion of chlorine and sodium. Thus 18 grms. of K_2O, taken in the form of phosphate or citrate, caused the loss of an extra 6 grms. of chloride of sodium (as well as 2 grms. of sodium in other forms), about one-half of the common salt which is contained in the 5 litres of a man's blood. And 18 grms. of potash is an amount much less than may be introduced with many important articles of vegetable diet, such as potatoes, which contain 20 to 28 grms. K_2O in each 1000 grms. of dehydrated material. "Having regard to the important part which salt plays in the organism (as in the formation of the digestive secretion, or in dissolving the globulins), even a small diminution may be prejudicial to certain functions, and may give rise to the need of recovering the loss."[2]

There are two other constituents of the food which need special consideration, namely, *iron* and *lime*.

The amount of iron which is egested is exceedingly small, and it may be expected therefrom that the amount present in the food under ordinary circumstances would also be small. Stockman has

[1] "Lectures," translated by Wooldridge, p. 119.

[2] Bunge, *op. cit.*, p. 121. The student is referred to Bunge's original publications ("Lectures" and *Ztschr. f. Biol.*, München, 1874, Bd. x.) for a full and very interesting discussion of this important subject.

shown that only about 10 mgrms. a day is ingested in an ordinary diet.[1] Of this amount, 1 mgrm. is egested by the urine, the remainder by the fæces. This cannot, however, represent all the iron metabolised, for the iron of the hæmoglobin of disintegrated blood corpuscles is retained, mainly by the liver, and is no doubt again built up into blood pigment. The nuclei of most cells, both animal and vegetable, contain appreciable quantities of iron, and in this form, and in the hæmoglobin of meat, it must occur in most food.[2] In both these cases it forms an integral part of the molecule of the proteid or nucleo-proteid, and under ordinary circumstances there is no inorganic iron, nor any iron salt of organic acid present in the diet. Such compounds of iron as are contained in nucleins—such, for instance, as the nuclein of the yolk of the egg—have been termed by Bunge *hæmatogens.* As this nuclein is the only iron-containing constituent of the yolk, it is clear that the hæmoglobin of the developing red corpuscles of the chick must derive its iron from it. It has further been shown by Socin, working in Bunge's laboratory,[3] that in mammals also hæmoglobin is manufactured when the only iron contained in the food is in the form of the same yolk-hæmatogen, and that the urine of animals (dogs) fed freely with egg yolk shows a marked increase in the amount of iron present.

It is noteworthy, as has been pointed out by Bunge, that the natural food of the infant, namely, milk, contains mere traces of iron, although the formation of hæmoglobin is actively proceeding. This is accounted for by the fact that the fœtus lays up a store of iron (in its liver and elsewhere) before birth, and gradually draws upon such store for the manufacture of hæmoglobin. Thus Bunge [4] found 18·2 mgrms. iron per 100 grms. body weight in a new-born rabbit, as compared with 3·2 mgrms. per 100 grms. in an animal twenty-four days old; and Zalesky,[5] four to nine times as much iron in the liver of a new-born puppy as in that of a full-grown dog.

In all other respects the composition of the ash of milk nearly corresponds with the composition of the ash of the sucking animal, as may be seen in the following table from Bunge, which gives the result of two experiments:—

	Puppy.		Milk of Bitch.	
	A.	*B.*	*A.*	*B.*
K_2O	11·42	8·50	14·98	10·70
Na_2O	10·64	8·20	8·80	6·10
CaO	29·52	35·8	27·24	34·40
MgO	1·82	1·60	1·54	1·50
Fe_2O_3 . . .	0·72	0·34	0·12	0·14
P_2O_5	39·42	39·80	34·22	37·50
Cl	8·35	7·30	16·90	12·40

[1] *Brit. Med. Journ.*, London, 1893, vol. i. pp. 881, 942 (contains the literature regarding iron absorption up to that date); *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 485; also, with Greig, *ibid.*, 1897, vol. xxi. p. 55.

[2] Bunge, *Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 49. For the microchemical evidence of the presence of iron in cell-nuclei, see Macallum, *Proc. Roy. Soc. London*, 1891, vol. l. p. 277; and *Quart. Journ. Micr. Sc.*, London, vol. xxxviii. p. 175. This will probably account for the fact that the fæces, which includes many disintegrated cells of the alimentary passages, sometimes shows a greater percentage of iron than is present in the food, although the secretions poured into the intestines only contain iron in minute amounts.

[3] *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 93 and 133.

[4] *Ibid.*, 1892, Bd. xvi. S. 177.

[5] *Ibid.*, 1886, Bd. x. S. 479 and 495.

In spite of the fact that it is the general experience of members of the medical profession, that the administration of iron salts promotes the formation of hæmoglobin in certain forms of anæmia (chlorosis), there is no satisfactory evidence that the administered iron enters into the formation of the newly-formed hæmoglobin, and it has even been denied that the alimentary canal is capable of absorbing iron given in such form. The experiments of Kunkel,[1] however, show that if iron salts are administered to animals along with their food, the blood, liver, spleen, and other organs exhibit an excess of iron over that of control animals. Hall[2] also obtained distinct evidence of iron absorption under like circumstances. When iron salts are injected subcutaneously into a vein, most of the iron appears at once in the urine, some is secreted into the intestine,[3] but some is stored in the liver and is only gradually eliminated. Experiments upon animals, in which the hæmatogens of Bunge have been removed from the food and replaced by iron salts, have been attempted,[4] but have presented serious difficulties.[5] Marfori,[6] however, working with Schmiedeberg, obtained a large amount of absorption of iron when given to dogs in artificial combination with albumin. Macallum also has shown that iron, both in organic and inorganic combination, is absorbed by the intestinal mucous membrane.[7]

Lime is taken in and assimilated by the organism, also in all probability in the form of organic compounds, probably with proteids.[8] It occurs in large amount in milk, but in most other forms of foodstuffs it is deficient as compared with other constituents of the ash; the leguminosæ contain more than most foodstuffs. The only food which has the same amount as milk is the yolk of egg, which should therefore always be given to children when milk is either not procurable or cannot be digested."[9]

The withholding of lime from the food of growing animals causes rickets;[10] but rickets may occur in children, in spite of their food containing an adequate amount of lime.[11] Probably, owing to abnormal conditions of nutrition, the lime is under these circumstances not assimilated.

In adult animals (pigeons), feeding with foods containing little or no lime has been found eventually to cause alterations in the bones, which become unusually brittle and thin (osteoporosis).[12]

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l. S. 11; 1895, Bd. lxi. S. 595.

[2] *Arch. f. Physiol.*, Leipzig, 1894, S. 456; and 1896, S. 49.

[3] Mayer, Diss., Dorpat, 1850, quoted by Bunge. Quincke (*Arch. f. Anat., Physiol. u. wissensch. Med.*, 1868, S. 150) failed to find it in an isolated portion of intestine with a Thiry fistula, but Macallum (*Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 268) obtained evidence of it in the crypts of Lieberkühn.

[4] Socin, *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 93; v. Hösslin, *Ztschr. f. Biol.*, München, 1882, Bd. xviii. S. 612; Hall, *Arch. f. Physiol.*, Leipzig, 1896, S. 142.

[5] Consult upon the subject, Bunge, "Lehrbuch," 1894, 3te Aufgabe, S. 83; and Wooldridge's translation; also Neumeister, "Lehrbuch," Jena, 1897, 2te Aufl., S. 382–392, where the subject is very fully treated and many more references to the literature will be found.

[6] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1892, Bd. xxix. S. 212.

[7] *Op. cit.*, 1894.

[8] Fokker, *Arch. f. d. ges. Physiol.*, Bonn, 1873, Bd. vii. S. 274.

[9] Bunge, "Lectures," Wooldridge's translation, p. 111.

[10] J. Forster, *Ztschr. f. Biol.*, München, 1873, Bd. ix. S. 369; and 1876, Bd. xii. S. 464; E. Voit, *ibid.*, 1880, Bd. xvi. S. 55; Baginsky, *Arch. f. Physiol.*, Leipzig, 1881, S. 357; and *Virchow's Archiv*, 1882, Bd. lxxxvii. S. 301; Seemann, *Ztschr. f. klin. Med.*, Berlin, 1882, Bd. v. S. 1 and 152.

[11] Rüdel, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxiii. S. 90; O. Vierordt, *Verhandl. d. xii. Cong. f. innere Med.*, Wiesbaden, 1893, S. 230.

[12] Chossat, *Compt. rend. Acad. d. sc.*, Paris, 1842, tome xiv. p. 451; C. Voit, *Ber. d. Vers. d. Naturf. z. München*, 1877, S. 243; Art. "Ernährung" in Hermann's "Handbuch," Bd. vi. S. 379; the earlier literature of the subject will be found in this article.

Metabolism during Inanition.

The problems of metabolism naturally subdivide themselves into those which concern the fate of the foodstuffs after they are absorbed and before they reach the tissues, and those which concern the fate of the stuffs which form the tissues, or which undergo changes within and by the agency of the tissues. The simplest condition of metabolism is therefore obtained when food is altogether withheld, as under these circumstances we have only to determine the changes which occur in the bodystuffs. On this account a very large amount of attention has been paid, both recently and previously, to the changes which occur in the tissues, as evidenced by the excreta during inanition in animals and man.

There is one main fact which comes out in all experiments on inanition, namely, that in spite of the withholding of food, all the excretions continue, not certainly to their normal amount, but at least to a considerable extent. This is even the case with the fæces which, in the absence of food, might be expected not to be formed. But, as a matter of fact, it is found that, during starvation, animals pass, if not every day, at least every two or three days, a fairly regular amount. This is composed of mucus and of inspissated digestive juices, a good deal altered in their composition, together with epithelial cells and other débris. Urine is also regularly passed during a period of inanition. The secretion of the skin is given off; carbon dioxide and water continue to be exhaled from the lungs; and in consequence of all these losses from the body the animal gradually loses in weight. The greatest proportionate amount lost is always during the first day of a fasting period. This is owing to the fact that the products of metabolism of the proteid food previously absorbed and that still within the alimentary canal are then got rid of. But after the first day or two it is found that the loss in weight is pretty definite, and nearly regular from day to day, and that fairly regular, or at least only gradually decreasing, amounts of the various excreta are lost daily.[1] Thus Voit,[2] experimenting upon a cat, found that about 4 to 5 grms. of urea were passed each day, representing a loss of tissue of from 25 to 30 grms., and this with great regularity until the twelfth day, when there was a marked rise in the amount of urea eliminated. And similar results have been obtained both with other animals and men in a condition of inanition.

The time at which this regular daily loss of nitrogen begins, depends upon the previous condition of nourishment. Thus, in a dog experimented upon by Voit, three series of experiments were made, each extending over eight days of total deprivation of food. The animal had received before the first series, 2500 grms. of flesh daily; before the second, 1500 grms.; and before the third, a mixed diet with relatively little proteid. The results obtained are shown in the table on p. 888. It will be seen that the regular loss begins at once in the third series, but not until the fifth day in the first series, in which the animal received most proteid during the previous period. The actual amount of proteid excreted per diem and per kilo. bodyweight was found by Voit

[1] The amount of *fat* metabolised in the dog was found by Pettenkofer and Voit to be *less* during the first days than during the subsequent period.

[2] Hermann's "Handbuch," Bd. vi.

Urea Excretion in Grammes per Diem.

Day of Inanition.	Series I.	Series II.	Series III.
1	60·1	26·5	13·8
2	24·9	18·6	11·5
3	19·1	15·7	10·2
4	17·3	14·9	12·2
5	12·3	14·8	12·1
6	13·3	12·8	12·6
7	12·5	12·9	11·3
8	10·1	12·1	10·7

to vary greatly with different dogs; small animals metabolise more proteid per kilo. than large; lean animals more than fat. Small dogs have a larger proportionate surface, and relatively a smaller amount of body-fat.[1]

Many similar observations have been made on fasting men. One of these (Cetti) was under observation at different times and by different observers. His weight was about 57 kilos. The amount of urea excreted per diem, during the first ten days of fasting, was a little over 20 grms., equivalent to from 10 to 11 grms. N. Another (younger) man, weighing about 60 kilos., was also found by I. Munk to excrete per diem, during the first ten days of fasting, about 11 grms. N, representing an average loss per diem of about 70 grms. proteid. In these cases there was but little body-fat. In other individuals, in which there was abundance of body-fat, the N excreted has been found to be much less. Thus Succi (weight 63 kilos. at beginning, 52 kilos. at end of period) was found by Luciani, during a thirty days' fast, to excrete on the tenth day 6·7 grms.; on the twentieth, 4·3 grms.; and on the last day 3·2 grms. N; and Jacques (62 kilos.), observed by Noël Paton and Stockman, gave an average daily loss of 5·29 N. Praussnitz determined the amount of N excreted by ten persons during the second day of fasting, and found the average, for a man weighing about 70 kilos., to be 13·7 grms., equivalent to a loss of 90 grms. proteid per diem, or about 1·2 grms. per kilo. body weight. This may therefore be regarded as representing the amount which it is absolutely necessary to supply in the food, for the maintenance of nitrogenous equilibrium.

In herbivora there may be an actual increase in the nitrogenous excreta at the beginning of a starvation period, instead of a diminution; due to the fact that, under these circumstances, such animals, being reduced to living upon their tissues, become practically carnivorous. As in carnivora, such increase may become greater towards the end of inanition, in consequence of the exhaustion of the fat of the body, and an increased destruction of the tissue proteids.[2]

Now, the amount of urea in the urine during a fasting period of not too long duration is probably a definite measure of the necessary destruction of tissue proteid which goes on within the body, and it may therefore be taken as a result of such experiments, that the amount of this metabolism is fairly constant. Such destruction occurs in spite of

[1] Rubner, *Ztschr. f. Biol.*, München, 1883, Bd. xix. S. 535.

[2] Rubner, *ibid.*, 1881, Bd. xvii. S. 214; Heymans, *Bull. Acad. roy. d. sc. de Belg.*, Bruxelles, 1896, p. 38.

the fact that there is still plenty of non-nitrogenous material (fat) able to be drawn upon. The sudden increase which is sometimes met with after a prolonged period of starvation is due no doubt to the fact that by this time the non-proteid materials of the body, which have been up to that time used for the production of energy by their oxidation, are now practically exhausted, and the whole energy and heat of the body must necessarily be derived from the tissues themselves; since these are composed essentially of proteid, there is a considerable rise of proteid metabolism.

The carbon dioxide exhaled from the lungs during starvation continues to be given off in proportion to the weight of the body, to the work done, and in inverse proportion to the temperature of the environment. In a man weighing 71 kilos., Pettenkofer and Voit found that during the first day of fasting 201·3 grms. C were given off by the respiration, and 5·8 grms. by the urine, in which also 12·5 grms. N was eliminated. This corresponded to a loss of 78 grms. proteid (370 grms. flesh) and 215 grms. fat. The same man was found by Pettenkofer and Voit to lose, when working on the first day of fasting, 75 grms. proteid (478 grms. flesh) and 380 grms. fat. The amount of oxygen taken in in the two cases was 760 and 1072 grms. respectively, and the amount of water exhaled 889 and 1777 grms. Ranke found on the second day of fasting, in a fat subject weighing about 70 kilos., 8 grms. N and 3·7 grms. C in the urine, and 180·9 grms. C given off by the lungs; corresponding to 50 grms. proteid (235 grms. flesh) and 204 grms. fat.

For a considerable time, as a result of the oxidation of fat and body proteid, the temperature of a fasting animal is maintained to about its normal amount. Towards the end, however, of starvation, the temperature begins to sink, and finally rapidly falls, the meaning of this being that the animal has now practically exhausted all the nutriment which it can take from the tissues, and that the amount of oxidation has become reduced, so that the temperature is no longer capable of being maintained at normal. The change is also, in part, doubtless due to the fact that the heat regulating functions of the nervous system are beginning to break down in consequence of the deficiency of nutriment. It has been suggested that an animal dying of starvation practically dies of cold; and it is undoubtedly true that the life of a starved animal can be prolonged considerably by the employment of artificial warmth, since this diminishes the amount of oxidation necessary for maintaining the animal heat, and thus economises the energy-producing substances within the body; but it is, of course, not possible for the artificial warming of an animal to prolong life to any great extent under circumstances of complete deprivation of food.

Numerous experiments have been made to determine the amount of loss of the several organs and tissues of the body which have occurred during starvation, and also the relative composition of such tissues and organs as compared with those of a well-nourished animal. All such experiments tend to show that the most essential organs of the body, such as the heart and nervous system, live during a period of starvation at the expense of the other tissues.[1]

[1] Bidder and Schmidt, "Verdauungssäfte u. Stoffwechsel," 1852; Bischoff and Voit, "Die Gesetze der Ernährung des Fleischfressers," 1860; Pettenkofer and Voit, *Ztschr. f. Biol.*, München, Bde. ii. and v.; J. Ranke, "Die Ernährung des Menschen," 1876; Voit, "Ernährung," Hermann's "Handbuch," 1881, Bd. vi.

The first substances to disappear, as may well be supposed, are those which are least essential to the maintenance of life, and we find accordingly that the adipose tissue first begins to lose weight. Finally, at the end of starvation, 90 per cent., or more, of the fats of the body (except the fatty substances which are found in the nervous system) have disappeared. At the same time the glycogen which may have been stored in the liver and muscles also begins to disappear; but it is a long while, in some animals, before the last traces of it are used up, especially the glycogen of muscle. Certain of the organs especially become diminished in weight. Among these the first to show a falling off are the spleen and the glandular organs, especially those concerned in digestion. Since there is very little secretion going on, these are not called upon to exercise their normal functions. Next follows marked diminution in the amount of the muscular substance, and this it is, no doubt, which accounts for the muscular weakness which manifests itself. When all the less essential organs have contributed as much as appears possible to the maintenance of the normal condition of the blood, in order that it may sufficiently nourish the most essential tissues, the latter, namely, the heart and those of the nervous system, might next be expected to contribute their quota. Apparently, as soon as this call is made, they fail to respond to it, and the result is that death speedily supervenes.

Voit gives the following percentage loss for the several tissues and organs in a cat killed after thirteen days' deprivation of food:—

	In 100 Parts of Fresh Organs.	In 100 Parts of Dry Organs.
Adipose tissue	97	...
Spleen	67	63
Liver	54	57
Testes	40	...
Muscles	31	30
Blood	27	18
Kidneys	26	21
Integument	21	...
Lungs	18	19
Intestines	18	...
Pancreas	17	...
Bones	14	...
Heart	3	...
Central nervous system	3	0

Tominaga[1] has determined (by Kjehldal's method) the amount of N lost from the several organs during a prolonged starvation period in rats and rabbits, as follows:—

Organ.	Rat.	Rabbit.
Spleen	98·48	67·06
Stomach and intestines	59·47	26·80
Muscles	35·98	18·59
Heart	18·01	22·74
Brain	11·79	29·13
Liver	9·69	57·60
Kidneys	3·48	24·80

The discrepancies in these results, both as compared with one another and as compared with the loss in the dry organs as determined by Voit, are so considerable, that they cannot be accepted without confirmation.

[1] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, Bd. vii. S. 381.

The literature of the subject, since the article by Voit in Hermann's "Handbuch" (1881), will be found mainly in the memoirs noted below.[1]

Nutrition with a purely Proteid Diet.

Under the circumstances we have been considering, namely, complete deprivation of food, the nitrogen excreted must come from the nitrogen of the tissues, and it might be supposed that if we supply a starving animal with food containing the exact amount of nitrogen (in the form of proteid) which it is losing, we should be able to entirely prevent such waste of the tissues, and that any loss then occurring would arise solely from non-proteid substances. This, however, is not the case. For if this experiment is performed, it is found that the animal loses more nitrogen than we give it. The whole of the nitrogen of the added proteid appears in the urine as urea, and in addition there is a certain amount, although not as much as during complete starvation, of tissue nitrogen still present in the urine. In order to keep up nitrogenous equilibrium, Voit found that it was necessary to give two and a half times as much proteid as the animal had metabolised during fasting. This result, which is at first sight somewhat unexpected, is due to the fact that the ingestion of proteid food directly excites the tissues to increased metabolic activity, so that tissue proteid itself still becomes split up and oxidised.

How and why the activity of the living tissues is thus stimulated by increased proteid pabulum is a problem as to which we are entirely in the dark. Non-proteid substances do not produce this effect. On the contrary, the giving of gelatin, carbohydrates, and fat has, as we have seen, a sparing effect upon proteid metabolism, and tends to diminish the amount of tissue proteid which is becoming broken down. This is also shown very conclusively in Voit's experiments on dogs which had been kept in a condition of N-equilibrium with proteid food. The condition of N-equilibrium could be produced with a far smaller amount of proteid, provided that for the amount removed an adequate quantity of fat or carbohydrate was added to the diet.[2]

If to a starving animal, instead of what would appear to be just a sufficient amount of proteid, an excess be given, a point is at length reached at which the building-up process exceeds the breaking-down, and the tissues, and therefore the body generally, gain in weight. This increase in body weight, due to the laying on of tissue, proceeds to a certain point with any constant amount of added proteid, until a balance between the N laid on and the N lost is struck, when a condition of N-equilibrium is again obtained. A further increase of

[1] Luciani, "Fisiol. d. digiuno," German translation, "Das Hunger," 1889; Richet, "L'inanition," Travaux, 1893, tome ii.; Tucsek, *Centralbl. f. d. med. Wissensch.*, Berlin, 1885, S. 69; Lehmann, Müller, Senator, Zuntz, I. Munk, and others, *Berl. klin. Wchnschr.*, 1887, S. 425; and *Virchow's Archiv*, 1893, Bd. cxxxi., Suppl.-Heft; I. Munk, *Centralbl. f. d. med. Wissensch.*, Berlin, 1889, S. 833; Noël Paton and Stockman, *Proc. Roy. Soc. Edin.*, 1889, p. 121; Praussnitz, *München. med. Wchnschr.*, 1891, No. 18; and *Ztschr. f. Biol.*, München, 1893, Bd. xi. S. 151; R. May, *ibid.*, 1893, Bd. xii. S. 29; I. Munk, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 309; Johansson, Landgren, Sondén and Tigerstedt, *Skandin. Arch. f. Physiol.*, Leipzig, 1896, Bd. vii. S. 29; C. Voit, *Ztschr. f. Biol.*, München, 1894, Bd. xxx. S. 510 (comparison of weight of organs in well-nourished and starved dogs). See also on this subject, Lukjanow, *Ztschr. f. physiol. Chem.*, Strassburg, 1889, Bd. xiii. S. 339.

[2] Voit, *op. cit.*

proteid food will now again produce an increase of tissue and of body weight, until again a condition of N-equilibrium is established. And this may apparently be carried up to the limit of the power of digestion of the animal for proteid food, so that ultimately fifteen times as much proteid may be metabolised as in the condition of inanition.[1] On the other hand, diminution of the amount of proteid food tends in the same way to gradually establish N-equilibrium on a lower level, and with a diminished body weight; the animal losing flesh until such equilibrium becomes established, and then maintaining itself, provided the N ingested be constant, at a constant but lower level of N-equilibrium. In short, "N-equilibrium is possible with the most different amounts of proteid in the food."[2]

The fact that the amount of urea excreted is directly dependent upon the amount of proteid ingested, is well illustrated by the following observations of Voit upon a dog fed on lean meat; the numbers are grms.:—

Meat per diem	.	300	600	900	1200	1500	1800	2000	2500
Urea per diem	.	32	49	68	88	106	128	144	173

About 80–85 per cent. of the ingested proteid is usually oxidated and eliminated, and only about 15–20 per cent. is laid on.

On the Building-up and Breaking-down of the Bodystuffs.

The food of animals consists, besides water and a certain amount of inorganic salts, of organic constituents, nitrogenous (some of which must be proteid) and non-nitrogenous. The food of the higher plants, on the other hand, consists normally of inorganic materials, some of which must be nitrogenous; and, as has been long recognised, plants have the power of building up from these materials complex organic substances, such as proteids, carbohydrates, and fats, whereas animals have not this power; the materials built up by plants serving as the food of animals. Hence arose the belief that it was an essential difference between the plant and animal organisation, that the one possessed extensive powers of effecting syntheses, whereas the other had practically no powers of synthesis, but must receive its materials already synthetised, either directly from plants or indirectly from plants through the bodies of other animals, such materials being subsequently broken down into simpler materials, which, after being oxidised within the tissues, are got rid of in such simple forms as urea, water, carbon dioxide, and salts.

These views have undergone considerable modification of late years, since we are now familiar with numerous instances of syntheses occurring in animals. The first well-established case of the kind was determined by Wöhler in 1824. Wöhler found that when benzoic acid is taken with the food, it appears as hippuric acid in the urine. Now, hippuric acid is formed synthetically from benzoic acid and glycine.

[1] C. Voit, Hermann's "Handbuch," Bd. vi. S. 105. Voit's dog, weighing 35 kilos., was able to maintain N-equilibrium with as little as 500 and as much as 2500 grms. flesh, containing 548 grms. dry proteid. With larger amounts than this, digestion was interfered with. The same fact is still more strikingly shown by the experiments of Pflüger, who kept a large dog in a condition of nitrogenous equilibrium on an almost exclusively proteid diet. A man weighing 70 kilos. is, as a rule, unable to digest more than 1500 grms. of lean meat per diem.

[2] C. Voit, *loc. cit.*, S. 111.

It is produced when these two substances are allowed to act upon one another at a high temperature, and under pressure, as when they are heated together for some hours in a glass tube to a temperature of 160° C., or more simply by heating monochloracetic acid with benzamide:—

$$\underset{\text{(benzamide)}}{C_6H_5CO.NH_2} + \underset{\text{(monochloracetic acid)}}{CH_2Cl.COOH} = \underset{\text{(hippuric acid)}}{(C_6H_5CO)NH.CH_2.COOH} + HCl$$

This synthesis of hippuric acid *in vitro* was speedily followed by that of urea (Wöhler, 1828).

The synthesis of hippuric acid was proved by Bunge and Schmiedeberg to occur in dogs exclusively in the kidney, and may be produced even at the temperature of the room, by passing oxygenated blood containing benzoic acid, or a benzoate, and glycine through the blood vessels of the organ, or even by allowing such blood to stand for a while in contact with the minced kidney of a fresh-killed animal. When, however, the kidney cells are destroyed, as by being pounded with sand in a mortar, no hippuric acid is produced. If benzoic acid be given by the mouth, hippuric acid appears in the urine; the glycine for the synthesis is furnished by the tissues. If the kidneys are previously extirpated, no hippuric acid is found in any of the organs after the exhibition of benzoic acid; but if the ureters are merely ligatured, hippuric acid is found in abundance.

In frogs and rabbits the synthesis of hippuric acid is not confined to the kidneys, but is found to occur after the extirpation of these organs.[1]

Other syntheses besides that of hippuric acid, which are known to occur in the animal body, are that of urea in the liver, from ammonium carbonate and ammonium carbamate; that of uric acid in the bird's liver, also from ammonia compounds; that of glycogen, from glucose in the liver, and also in muscles and in many other tissues; that of proteids, from peptones in the mucous membrane of the alimentary canal; that of fats, from fatty acids and glycerin in the intestinal mucous membrane; that of fats from carbohydrates, or from the elements of the broken-down carbohydrate molecule; and also, in all probability, that of fats from the non-nitrogenous moiety of the broken-down proteid molecule. It is clear from these instances that the importance of syntheses in the animal economy cannot be overrated, and although the most striking feature in animal metabolism is the breaking down of complex substances into others of more simple form, yet even in the case of these broken-down products there is frequently a subsequent synthesis before they are got rid of from the body. Instances of this occur in the case of several urinary products, such as hippuric acid, urea, and uric acid.[2]

As Bunge[3] remarks: "There are two reasons why these synthetic processes in the animal body have excited the interest of physiologists and chemists. In the first place, they were in contradiction to the long dominant doctrine of Liebig, as to the contrast between the metabolic processes in plants and animals;[4] and, in the

[1] Bunge and Schmiedeberg, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1876, Bd. vi. S. 233; Hoffman, *ibid.*, 1877, Bd. vii. S. 239; Kochs, *Arch. f. d. ges. Physiol.*, Bonn, 1879, Bd. xx. S. 64; Salomon., *Ztschr. f. physiol. Chem.*, Strassburg, Bd. iii. S. 365.

[2] On the importance of synthetic processes in animal metabolism, see Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 144.

[3] "Lehrbuch," 1894, S. 288.

[4] Nevertheless, the main distinction propounded by Liebig, that most plants are able to obtain their nitrogen, and to build it up into proteid from inorganic materials, whereas animals do not possess this power, still holds good.

second place, the methods of synthesis in animal (and vegetable) organisms are still an unsolved problem, in spite of the fact that it is the rapid progress in our knowledge of the syntheses of organic combinations which constitutes the greatest triumph of modern chemistry. Chemists are already able artificially to build up atom for atom out of their elements a series of organic compounds, some of a very complicated nature. We no longer doubt that all the rest, even the most complex, will be thus produced. Nevertheless the processes employed in no way represent the synthetic processes of the living cell, for all artificial syntheses can only be achieved by the application of forces and agents which can never play a part in vital processes, such as extreme pressure, high temperature, concentrated mineral acids, and free chlorine—agents which are immediately fatal to any living cell."

It must nevertheless be admitted, in spite of the numerous instances of syntheses of organic compounds which have accumulated of late years, that, so far as the formation of bioplasm is concerned, the only material from which the animal organism is capable of forming it is proteid, and this proteid must be present as such in the food. No doubt the ultimate change of the circulatory or blood proteids to the proteid of bioplasm must depend upon a special synthesis, but we are necessarily completely ignorant as to the manner in which such synthesis occurs, since we are ignorant of the actual chemical constitution of both living tissue and dead proteid.

With respect to the breaking-down of the bodystuffs in the process of metabolism, there are reasons for believing that this consists of two phases, namely, a splitting of the complex molecules into simpler molecules, and an oxidation of some or all of the simpler substances thus arising. It is probable that in the metabolism of proteid these two phases usually, if not invariably, occur at different times, and even in different places in the body; for example, the materials derived from the splitting up of the metabolised proteids of muscle do not all leave the muscle in a fully oxidated condition, but are, in part at least, in the form of oxidisable substances, such as lactic acid. Doubtless, in the formation of the ultimate products, oxidation is the prominent feature, for these products, in the form in which they leave the body, are, as compared with the materials that enter the tissues, unquestionably in a condition of oxidation, in some cases of complete oxidation. There is, however, no distinct evidence that the process of splitting of the complex molecules is necessarily immediately combined with that of oxidation. On the other hand, there is reason to think that such splitting may occur without immediate oxidation; for example, the splitting of proteids, which are taken in the food, into urea and non-nitrogenous substances. For, in a dog fed with proteid, the urea was found by Feder to make its appearance in the urine within fourteen hours after feeding, whereas the removal of the remainder of the proteid molecule in the form of carbon dioxide and water did not occur for twenty-four hours after, so that the splitting of the proteid molecule must have occurred at one time, and its complete oxidation at another.[1]

It is found that any conditions which tend to diminish the normal oxidations of the body generally, or of the individual tissues (such as the ingestion of prussic acid or the cutting off or diminution of the arterial supply to an organ), cause such substances as lactic acid and dextrose, which are probably products of proteid and carbohydrate

[1] C. Voit, *Ztschr. f. Biol.*, München, 1891–2, Bd. xxviii. S. 292.

metabolism respectively, to appear in larger amount than usual in the blood, and to become excreted in the urine.[1]

Relative Metabolic Activity of the Tissues and Organs.

Before we trace the fate of the foodstuffs in the body, it is important we should have an idea of the relative metabolic activity of the tissues, since all essential changes which contribute to the production of the energy of the body occur within the tissues.

It was at one time believed that the blood was the seat of important oxidation processes; but whilst it cannot be denied that a certain amount of oxidation may occur in the blood, as shown by the rapid diminution in the oxygen of the oxyhæmoglobin, on allowing blood to stand in a closed vessel,[2] it is certain that by far the greatest part of the oxidations in the body occurs in the tissues, and especially in the muscles. It was found by Pflüger, that frogs whose blood had been wholly replaced by salt solution took in just as much oxygen, and gave off just as much CO_2, as normal animals.[3] Moreover, Pembrey and Gürber found hardly any alteration in the oxidation processes in rabbits which had been deprived of a large proportion of their blood.[4]

Placing the tissues in order of relative activity, the muscles must take the first place; next to these the secreting glands; and next to these the tissues of the nervous system, especially the grey matter. Last in the scale come the skeletal tissues, which, performing as they do a passive function, may be assumed to exhibit comparatively little metabolic activity. With regard to the most active of the tissues, namely, the muscles and the cells of secreting glands, we may note, in passing, that their chemical composition is by no means identical. The most prominent organic material in muscular tissue is native proteid of the globulin class, whereas the most prominent organic materials in the living tissue of gland cells are nucleo-proteids. This distinction, though frequently ignored, is one of considerable importance, for the nucleo-proteids have a constitution more complex than that of proteids, consisting as they do of a combination of proteid with phosphorus-containing substances, which yield as products of decomposition, xanthine bases, nucleins, paranucleins, and phosphoric acid, and some of them, at all events, a carbohydrate (see pp. 66, 67).[5]

There can be very little doubt that the greater part of the oxidation of the body occurs in the muscles. The formation of heat can, in fact, be shown to be mainly due to the chemical activity of the muscles, an activity called into play under the influence of the nervous system;

[1] Zillesen, *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 387; Araki, *ibid.*, 1891–4, Bde. xv., xvi., xvii., xix.

[2] The disappearance of the oxygen of oxyhæmoglobin which occurs in blood on standing, has been ascribed to the presence of hypothetical substances, to which the term "reducing substances" has been applied. No chemical substances having such a reducing power have, however, been either isolated from blood or chemically investigated. Moreover, the reduction of oxyhæmoglobin in blood on standing, may be due to its oxygen being removed by the bioplasm both of the white corpuscles and of putrefactive bacteria, which rapidly begin to appear and multiply in drawn blood. Reduction even occurs with solutions of pure crystallized oxyhæmoglobin hermetically sealed in glass tubes.

[3] *Arch. f. d. ges. Physiol.*, Bonn, Bd. x. S. 251; see also Œrtmann, *ibid.*, 1877, Bd. xv. S. 382.

[4] *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 449.

[5] In connection with this question, the possibility must not be forgotten that even ordinary proteids may have a carbohydrate nucleus in their molecule (cf. p. 64).

and the greater the amount of muscular activity the greater the amount of oxidised materials in the form of carbonic acid and water that are formed and got rid of from the body. It is probable that the oxidation processes which occur in gland cells are by no means so active, for although a gland when stimulated to activity receives a larger amount of oxygenated blood, yet a considerable amount of the oxygen of that blood simply passes through the capillaries without being absorbed, so much so, in fact, that, as noted by Bernard, the blood of the veins of the salivary glands during stimulation of their cranial nerves flows almost as bright red as that of an artery. And in confirmation of this we find that the largest gland in the body, the liver, is supplied with a relatively small amount of arterial blood, and that almost the whole of its metabolic activity is carried on with blood which already has passed through the intestinal capillaries, and which has thereby been deprived of a large part of its oxygen. Further, it was noted by Ludwig that the saliva flowing from the duct of the submaxillary gland contains more oxygen, than is dissolved in the plasma of the arterial blood, an indication that the cells of the salivary glands cannot be greedy of oxygen since they pass oxygen out along with the secretion rather than retaining it for the formation of carbon dioxide and water. The salivary glands, moreover, have been shown by the recent careful observations of Bayliss and Hill[1] not to produce any appreciable amount of heat; and although it is stated that the blood flowing through the liver is the warmest blood in the body,[2] and warmer than that flowing through the muscles, it must be borne in mind that it is almost impossible to measure exactly the normal temperature of the blood flowing from the muscles, because the operation necessary for observing the temperature of such blood would tend to expose it to loss of heat.[3]

In conformity with the conclusion that the muscles are the organs which possess by far the greatest amount of metabolic activity, it has been estimated that the muscular tissues contain about one-fourth of the whole blood of the body. The liver, which has important special functions to perform in metabolism—functions which are, however, probably in large measure independent of oxidation—contains another fourth of the blood, one-fourth is employed in keeping full the larger arteries and veins, and all the rest of the body put together has for its capillary supply only the remaining fourth. It is clear, then, that in all observations and experiments upon the metabolism of the body, the metabolism of the muscles must occupy a prominent place.

Nitrogenous Metabolism in the Tissues.

Of the proteids of the body Voit distinguishes two kinds—(1) Those which form an integral part of the living substance or bioplasm, and (2) those which occur in the tissue juice and in contact with the bioplasm, but which are not to be regarded as forming an integral part of that substance itself. To this latter kind he has given the name of "circu-

[1] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 351. Previously to this work, it had been accepted, on the authority of Ludwig and others, that the secretion of saliva is accompanied by a marked production of heat within the submaxillary gland.

[2] See p. 826. Waymouth Reid was unable to find any effect on the temperature of the liver as the result of stimulating the splanchnic and vagi nerves ("Proc. Phys. Soc.," 1895, p. xxxi., in *Journ. Physiol.*, Cambridge and London, vol. xviii.).

[3] In his experiments upon the gaseous exchange in blood perfused through "surviving" mammalian muscle, v. Frey found that the blood leaving the muscle was slightly warmer than that entering it (*Arch. f. Physiol.*, Leipzig, 1885, p. 559).

lating proteid," while the proteid which is assumed to actually form the living substance of the tissues is termed by Voit, *Organeiweiss*, which may be rendered in English by "organ- or tissue-proteid."[1] If the term "circulating proteid" be used to include the proteids of the blood and lymph as well as those which occur in the actual interstices, if any, of the bioplasm, no exception can be taken to it, but if it is used, as has been sometimes done by Voit, in a restricted sense, merely to indicate proteid material which is interpolated amongst the molecules of the proteid forming the bioplasm, without itself actually constituting part of that substance, it must be admitted with Pflüger[2] that such employment of the term can only be misleading. Using, however, the term circulating or unorganised proteid in the wider sense, there are still two possibilities open as to the manner in which the proteids of the body undergo metabolic changes—(1) We may assume that the circulating proteid, reaching the tissues and becoming imbibed by them, must be completely incorporated and built up into them before it is split up and oxidised; or (2) it is open to us to suppose that the unorganised proteid may be split up and oxidised outside the actual molecules of the organised proteid of the living substance, but as a consequence of the action of that substance. In the one case we may suppose it to produce a direct formation or building up of bioplasm—a transformation, in fact, of unorganised into organised proteid; in the other case, as undergoing contact changes by the action of the bioplasm, much in the same way as contact changes are brought about by organised ferments.

One reason for believing that the circulating proteid only becomes in part built up into the material of the bioplasm, is derived from the following observation (Voit). If, to an animal kept upon a diet consisting of non-proteid food (fat), gelatin is given in an amount sufficient to replace a caloric equivalent of such non-proteid material, it is found that, reckoning for the amount of nitrogen due to the metabolised gelatin, which always appears in full as urea, there is less nitrogen given off from the body than before; that is to say, there is less tissue substance broken down. But in the total absence of nitrogenous food there is a definite amount of body proteid metabolised; and since, when gelatin is given, it is metabolised instead of part of this proteid, although it cannot itself be built up into tissue substance (p. 878), it must be assumed that the gelatin has taken the place of proteid which, although in such intimate contact with the bioplasm as to become metabolised under its influence, did not actually form bioplasm. It may further be argued that the rapidity with which metabolic changes in proteids occur within the body, and the large amount of such metabolism, when excess of proteid is taken as food, render it improbable that all metamorphosed proteid has been built up to form bioplasm.

[1] C. Voit, "Die Ernährung," Hermann's "Handbuch," Bd. vi. S. 301. The terms "organised" and "unorganised" proteid are preferable to "tissue-" and "circulating-" proteid, which have been used at different times in different senses. In earlier publications (*Ztschr. f. Biol.*, München, 1874, Bd. x.) Voit included the proteids of blood plasma under the designation "Organeiweiss," founding this view upon the fact that, as the experiments of Tschiriew (*Ber. d. k. sächs. Gesellsch. d. Wissensch.*, 1874, S. 411) and Forster (*Sitzungsb. d. k.-bayer. Akad. d. Wissensch. zu München*, 1875, S. 206) seemed to show, transfusion of blood does not increase the proteid metabolism of the body. Pflüger, however (*loc. cit., infra*, pp. 362 *et seq.*) has shown that the results of Tschiriew and Forster are capable of a diametrically opposite interpretation.

[2] *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 333.

Voit has drawn a much sharper distinction between the organised and unorganised (tissue and circulating) proteid than that above indicated. He denies that tissue proteid can as such undergo metabolism, even in inanition. According to his view, it must first be dissolved up and take the form of circulating proteid, and be carried in this form to other tissues (*e.g.* from the muscles to the heart and nervous system), to be metabolised as circulating proteid in these.[1] This view is, however, difficult to reconcile with the supposition that there is no *chemical* difference between the two forms of proteid,[2] for if there is no such difference, it is not clear why the proteid should not become metabolised in the tissues themselves, but should need to be conveyed outside them before undergoing metabolic changes. Moreover, it is entirely inconsistent with the experiments of Oertmann and Pflüger, of Pembrey and Gürber, and of Schöndorff (with Pflüger), which will be subsequently referred to.

The arguments and experiments by which Voit has endeavoured to support his position are, however, quite insufficient to carry conviction, and it must be regarded as having been rendered completely untenable by the experiments and criticisms of Pflüger.[3] A view exactly the contrary to that of Voit was taken by Liebig, and has been maintained by Hoppe-Seyler, and in a somewhat qualified form by Pflüger. According to this view, it is only organised proteid which can undergo metabolic changes—never unorganised. Unorganised proteid must therefore first be converted into organised before it is capable of metabolism; in other words, tissue bioplasm must be built up out of circulating proteids before these last, which then of course have become tissue proteids, can be broken down and oxidised. It is therefore denied that any metabolism of proteids can occur outside the actual molecules of the living substance—that, in short, there can be any contact action. It has, however, been shown in the case of yeast, that chemical action may take place outside the living cells, although under their direct agency, so that the possibility of metabolic changes occurring under the influence of, but outside, the actual molecules of the protoplasm of cells cannot be denied. Moreover, it is not probable that the non-proteid materials (fat, carbohydrate, gelatin) of the food become after assimilation built up into bioplasm, and although they are undoubtedly taken into cell protoplasm they can hardly be regarded as forming constituent parts of the molecules of its bioplasm. In this sense, therefore, they are outside, although in contact with, the bioplasm of the tissues; nevertheless they are found to undergo metabolic changes under the influence of that substance. It may, of course, be argued that they also are really built up into the living proteid molecule, and must be so before they can become metabolised, but there is absolutely no evidence that this is the case, or that fat or carbohydrate are necessary constituents of bioplasm.

The fat drops which we see embedded in the protoplasm of cells, are certainly not constituent parts of the bioplasm, although under its influence they undergo physical and chemical changes, and the same is the case with the glycogen clumps which can be seen in the liver cells, to say nothing of the starch, aleuron, and fat granules of vegetable cells. The phenomenon of contact change is in short too universal to be denied. Since this is so, the most reasonable view to be taken of the matter appears to be one which supposes that metabolism may occur both as a splitting-up and oxidation of the molecules of living tissue or bioplasm, and as a splitting-up and oxidation

[1] *Loc. cit.*, S. 303.

[2] "Ich will also nicht damit einen chemischen Unterschied bezeichnen, sondern zunächst nur einen Unterschied in dem Orte an dem es sich befindet. . . . Ein und dasselbe Molekül Eiweiss kann in einem bestimmten Momente Eiweiss des Blutplasmas, in einem nächsten Eiweiss der Ernährungsflussigkeit, in einem anderen Eiweiss der Lymphe oder auch Organeiweiss sein" (*loc. cit.*, S. 301).

[3] *Loc. cit.*

both of unorganised proteid and of non-proteid materials outside but in contact with the molecules of bioplasm. Such a view, which is in a sense intermediate between the extreme opinions advocated by Voit and Pflüger respectively, is consistent with all the known facts, and is more readily applicable to the phenomena, both of animal and vegetable metabolism, than the exclusive acceptance of either of those opinions.

Whether directly or indirectly, tissue proteid normally undergoes metabolism to the extent of about 1 per cent. of its substance per diem (Voit).

The proteids of the food are converted by digestion into albumoses and peptones; ultimately, probably, entirely into peptones. They are, however, not absorbed as peptones, for no peptones are found in the blood or chyle leaving the intestines. It is clear, therefore, that the process of assimilation or the reconversion of peptones into proteids must occur during their absorption, that is to say, in the substance of the mucous membrane. It must not be forgotten, however, that a certain amount of the proteid of food may possibly, as occurs *in vitro*, be broken down beyond the stage of peptone into simpler nitrogenous bodies, such as the amido-acids; and these, if their formation really occurs to any extent in the intestinal tract, would be absorbed as such into the portal blood and conveyed by it to the liver. Now we know that the addition of amido-acids to the blood which is allowed to circulate through the liver, as well as their administration with the food, causes an increase in the amount of urea in the blood after it has passed through that organ, and an increased excretion of urea by the kidneys.[1] From this it may be assumed that any amido-acids absorbed are converted by a process of synthesis (possibly preceded by a previous more complete breaking-down, into ammonia compounds) into urea. If this process of formation of amido-acids occurs at all in natural digestion, it is obviously a change by which the proteids of the food would not be directly serviceable for the production of tissue; and in this sense such conversion of peptones into amido-acids may be looked upon as a direct waste of proteid food. It is extremely improbable that such a change occurs to any extent in the normal organism, nor has the presence of these substances to any marked degree been determined in the normal intestinal contents. Moreover, as Bunge remarks, there is not sufficient carbon in the proteid molecule to permit of all the nitrogen issuing as amido-acids.[2] In any case, these bodies must probably be split up and oxidised into carbon dioxide and ammonia, and from these urea become formed by synthesis in the liver. We may therefore probably put aside as exceptional this mode of transformation of proteid into urea, and consider only the change which is undergone by the proteid which is actually assimilated.

With regard to the agents in the mucous membrane which produce the assimilation of proteids, that is to say the conversion of peptones into proteids, there can be very little doubt that the columnar epithelium occupies the first place. It is, however, difficult to prove that the change, which is one of synthesis and dehydration, does actually occur in these cells. We have chiefly analogy to guide us in coming to this conclusion. It can be definitely proved that a synthesis of fat does occur in them; and it is therefore probable that

[1] Schultzen and Nencki, *Ztschr. f. Biol.*, München, 1872, Bd. viii. S. 124; Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1879, Bd. iv. S. 100; v. Knieriem, *Ztschr. f. Biol.*, München, 1874, Bd. x. S. 279.

[2] "Lectures," p. 320.

other syntheses which accompany assimilation, such as the formation of proteids from peptones, must also occur within them. Hofmeister [1] has suggested that the leucocytes may also take an important part in determining the assimilation of the foodstuffs. They are present in great abundance in the intestinal mucous membrane, and especially those parts of that membrane where absorption proceeds most extensively; and they are also, it has been shown, greatly increased in number during the process of absorption. It is, however, difficult to obtain affirmative evidence upon this point, and since leucocytes elsewhere do not possess this power, it is improbable that they are the agents for such conversion in the intestine.

After assimilation the proteids are absorbed by the blood vessels of the intestinal mucous membrane. The evidence for this has been already given in the article on "Digestion and Absorption" (p. 433). If any proteids are taken up by the lacteals of the small intestine, they do not get into the thoracic duct,[2] but must be transferred to the blood vessels in passing through the mesenteric glands. At any rate we may assume that nearly the whole of the proteids are ultimately taken by the portal vein to the liver. The portal vein, therefore, contains the absorbed material derived from digestion and assimilation of proteid food; it must have, therefore (besides the ordinary constituents of blood plasma), an additional amount of serum albumin or of serum globulin, obtained by the transformation of the peptones into these materials; also extractives of the meat or other forms of proteid diet (which are absorbed equally by the blood vessels of the intestine), and in addition any products of further decomposition of peptones, such as the amido-acids, the possibility of the presence of which we have already discussed. But it must be borne in mind that the blood flow through the portal system is so large and rapid, that one could hardly expect these substances to be absorbed into it in such a proportion that it would be possible to detect by chemical means any appreciable difference of composition between the blood of the portal vein and that of the system generally, nor are there any satisfactory analyses directly showing such difference. Nevertheless there is a distinct physiological difference between the portal blood collected during absorption of food, and especially of proteid food, and the same blood collected during the intervals of digestion; for it has been shown that in the former case such blood, on being passed through the liver, shows an increased amount of urea, whereas in the latter case such an increase is not noticed. It is certain, at any rate, that the products of absorption and assimilation of proteid foods are carried to the liver, and, having traced them to this organ, we have next to consider—(1) Whether they are stored at all within it; (2) whether they undergo any change in passing through it.

Influence of the liver on proteid metabolism.—With regard to the possible storage of proteid in the liver, it is open to us to suppose that an excess of proteid material which is present in the portal blood as the result of the absorption of proteid food, might be temporarily taken up, at least in some measure, by the hepatic cells, and, after being

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1884–7, Bde. xix. S. 1; xx. S. 29; xxii. S. 306.

[2] Asher and Barbera found, however, in a dog a marked rise both in the amount of chyle and of the nitrogen of the chyle (estimated by Kjeldahl's method) during digestion of purely proteid food, given by a gastric fistula. The rise was greatest at the sixth hour after feeding, but there was a primary culmination at the second hour (*Centralbl. f. Physiol.*, Leipzig u. Wien, 1897, Bd. xi. S. 403).

stored within them for a time, passed on into the hepatic blood to reach the general circulation. There is, however, no clear evidence that such storage takes place in the liver, or that if it does the stored proteid undergoes any change within the liver cells.

When we examine the secretion of the liver, we find that it contains a considerable amount of nitrogenous organic material (bile salts), including a certain amount of sulphur in organic combination (taurine). These nitrogenous and sulphur-containing materials can only be derived from proteids, and since they are formed in greater amount during absorption of digested products than at other times, it might well be supposed that they may be formed, at least in part, from the absorbed products of proteid digestion. As against this conjecture, we cannot, however, fail to notice that the appearance of these nitrogenous and sulphur-containing materials in the bile salts is accompanied by a considerable amount of material in the form of bile pigments, which can only be derived from the hæmoglobin of the red blood corpuscles; and since hæmoglobin readily decomposes into hæmatin, which is probably the part directly converted, with elimination of iron, into bile pigment, and a proteid or proteids, it has been conjectured, and with some probability, that the bile acids are actually derived from this proteid part of the broken-down hæmoglobin molecule. The only direct evidence that we have of the breaking-down of blood corpuscles within the liver is derived from certain enumeration experiments, which appear to show that the number of blood corpuscles per cubic millimetre passing to the liver is greater than the number per cubic millimetre in the blood flowing from the liver.[1] This by itself is not very strong evidence, but it becomes stronger when we remember that the blood flowing from the liver may be expected to contain *less* water than that which reaches the liver, since there has passed away from it the water of the bile and also a large amount of lymph. Moreover, the constant presence of lecithin and cholesterin in the bile may well be associated with the destruction of red blood corpuscles, which contain, relatively, considerable amounts of these substances.

While, therefore, the presence of the bile acids is a clear indication of the breaking-down of proteid in the liver, their presence does not necessarily indicate that such proteid is derived from the blood plasma, but it is, on the whole, more probable that it arises from the hæmoglobin of blood corpuscles.[2]

The storage of glycogen in the liver, under circumstances when it can only be supposed to be formed from proteid, indicates another change which proteids may undergo in this organ. Such a formation of glycogen from proteid probably occurs hardly at all during absorption of a mixed meal, because the amount of carbohydrate absorbed from such a meal would be more than sufficient to account for the glycogen stored in the liver; but, in the absence of carbohydrate from the food, the proteids may become so split up that one portion of the proteid becomes converted into urea, or into materials which ultimately form urea, and the other portion, possibly by becoming first somewhat broken down and then again synthetised, into glycogen.

A similar conjecture may be made with regard to the fat which is

[1] Nicolaides, *Arch. de physiol. norm. et path.*, Paris, 1882, p. 531.

[2] According to Kunkel, taurine may be formed from proteids in the tissues generally, and carried to the liver, *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, 1875.

found in the liver cells during absorption. We must, it is true, assume that when fat is present in the food, the fat which occurs in the liver cells during absorption is derived from it; for the absorbed fat, after passing through the columnar epithelium cells, in which there is little doubt that it undergoes metabolic changes, gets into the thoracic duct, and so into the blood, in which it is carried to the liver and elsewhere. Nevertheless, in the absence of fat from the food, any fat which is found within the liver cells may be supposed to be obtained from proteid after the splitting off of the elements of the urea molecule; but we must suppose a considerable breaking-down and a re-synthesis to occur. In both carbohydrate and fat formation we must recognise the possibility of the preliminary splitting of the proteid molecule occurring elsewhere than in the liver, although there is no reason to suppose that the protoplasm of the hepatic cell does not possess, in common with protoplasm in general, the power to produce this change. The possibility of fat being formed from proteid is shown by the fact that in dogs subjected to a twelve-day period of inanition, and to which phosphorus is then administered, the liver contains from two to four times as much fat as in the normal animal—the amount of fat in the muscles being also greatly in excess of the normal amount.[1] This question of the formation of carbohydrate and of fat from proteid, both within the liver cells and elsewhere, will be considered later on.

The circumstances that in mammals urea, and in birds uric acid, occur in a larger proportion in the liver than in any other organ in the body, that there is an increase of urea in blood which has been passed through the liver, provided such blood is derived from an animal during absorption of proteid food, and that if blood containing certain ammonia compounds is passed through the liver it receives a very appreciable addition of urea,[2] all point to the fact, which is now unquestioned, that urea and uric acid are produced, if not exclusively, at all events mainly, in this organ.

But the urea which is found in the liver, and which is passed by the hepatic capillaries into the hepatic blood, although ultimately derived from the proteids of the food, is in all probability not to any appreciable extent immediately so derived. If this were to be the case, we should have, as with the formation of leucine and tyrosine in the intestine, so far as the tissues generally are concerned, a waste of nutritive material—a condition which is unlikely to obtain to any extent in the animal economy. It may therefore be taken for granted that the great part of the proteid which is absorbed from the intestine passes on through the hepatic veins into the general circulation, without being stored or at once modified in the liver; and since, after the absorption of any large amount of proteid from the alimentary canal, the relative and absolute amount of proteid in the blood and lymph is not materially altered, we may assume that the excess proteid is stored somewhere else.

The place of such storage is probably not far to seek. The fact that an increase of assimilated proteid in the blood rapidly increases the metabolism of muscles, points at once to such proteid passing into the

[1] Storch, Diss., Kjöbenhavn, 1865; *Deutsches Arch. f. klin. Med.*, Leipzig, 1867, Bd. ii. S. 264; Bauer, *Ztschr. f. Biol.*, München, 1871, Bd. vii. S. 63; 1878, Bd. xiv. S. 527; Caseneuve, *Rev. mens. de méd. et chir.*, Paris, 1880, tome iv. pp. 265, 444; Stolnikow, *Arch. f. Physiol.*, Leipzig, 1887, Suppl., S. 1.

[2] v. Schröder, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1882, Bd. xv. S. 364; *ibid.*, 1885, Bd. xix. S. 273; Salomon, *Virchow's Archiv*, 1884, Bd. xcvii. S. 149.

muscles; and since such increased metabolism continues during some time, the excess proteid must be supposed to be in the first instance stored within them.

The influence of assimilated proteids in increasing the metabolism of the tissues, and the manner in which such increased metabolism is brought about, has received the attention of many workers. According to the view held by Voit, which has been already referred to, such additional proteid is not built up into the bioplasm of the tissues, but passes into the tissues, and, by contact with the bioplasm, stimulates it to increased metabolism; such metabolism occurring, according to Voit, entirely in the circulating proteid, and outside, in a sense, the actual bioplasm. On the other hand, according to the view which has been strenuously supported by Pflüger, such excess of proteid is directly stored, not in the interstices of the bioplasm, but by being built up into its constitution; so that this substance grows at the expense of any excess of proteid pabulum which is brought to it by the circulating fluid, and such growth or increased nutrition of living substance in itself directly promotes an increased destruction.

That this view is, at least in part, correct, appears from the experiments by Schöndorff, which were carried out under Pflüger's direction.[1] Schöndorff perfused blood, taken from a dog which had been kept fasting for some days—(1) through the limbs, and then through the liver, of a well-nourished dog, which had been kept chiefly upon proteid food, and which was killed immediately before the experiment; (2) through the limbs, and then through the liver, of a dog which had been kept fasting for some days; and (3) blood, which was taken from a well-nourished dog, was passed through the limbs and liver of a fasting animal. In five experiments in which blood from a fasting animal was sent through the organs of a well-nourished dog, the urea of the perfused blood was increased by amounts varying from about a quarter to more than double its original quantity. Out of five experiments, in which the blood of a fasting animal was sent through the organs of a fasting animal, the amount of urea was diminished in two by 9·55 and 6·9 per cent., while in three it was hardly appreciably altered. In these cases, therefore, there was practically no proteid metabolism. In two experiments in which the blood of a well-nourished animal was sent through the organs of a fasting animal, the urea of the blood was diminished by 13·5 and 14·4 per cent. There was therefore also here no proteid metabolism, the diminution of the urea having been probably due to diffusion out of the blood into the tissues. That the increase which was obtained by passing the blood of a fasting animal through well-nourished organs was not due to the diffusion of pre-existing urea from the well-nourished liver, was determined by a control experiment, in which it was found that the amount of such diffusion was at most very small.[2]

These experiments show, according to Pflüger,[3] that the effect of increased proteid food has been to produce change in the bioplasm, directly causing this to grow and to become more active in its metabolism; whereas, on the other hand, a diminution of proteid food has produced the reverse change, namely, diminution in amount of bioplasm, with inactivity of proteid

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 420.

[2] The amount of urea in the blood of the starved dogs averaged 0·0348 per cent.; the maximum amount in the proteid-fed dogs, 0·1529 per cent.

[3] *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 408.

metabolism, even at a time when the tissues are temporarily supplied with circulating proteid from the blood of a well-nourished animal.

That a continuance of liberal proteid diet does produce an increased growth of muscular tissue, may also be looked upon as extremely probable, from the daily experience of athletes. As is well known, the diet upon which training is chiefly carried out consists very largely of proteid matter, the proteids of the food being in much larger proportion to the fats and carbohydrates than in the normal diet of untrained persons. It would appear likely that this, which is the result of the experience of many generations of trainers, must have a physiological basis, and that the effect of such excess of proteid in the diet must in itself not only cause an increase of the proteid metabolism, but also lead to the formation of actual tissue proteid. Under ordinary circumstances, however, whether the proteid which passes to the muscles is actually built up into their tissue, or whether it is simply included in the interstices of the living substance, it is not stored there for long; for it is found, after a meal containing much proteid, that within a few hours practically the whole of the proteid which has been absorbed is removed in the form of urea.

That the change in proteid which results in the formation of urea must *primarily* occur within the muscles, within which, as we have seen, the greater part of the oxidations of the body occur, there can be very little doubt. But there has always been this difficulty in connection with the question, that although urea is the ultimate product of proteid metabolism, the muscles practically contain either no urea or only a very small amount. An exception is, it is true, found in certain animals, *e.g.* the Elasmobranch fishes, the muscles of which contain a considerable amount of urea. But this is not the case with most animals, and it cannot be supposed that urea is formed to any appreciable amount in the muscles, especially since we know that by far the greatest amount is actually formed in the liver.

What precursor, therefore, of urea is formed in the muscles from the proteid which is metabolised within them? The nitrogenous substance which could best be supposed to be produced from the metabolism of proteids, is creatine, since this is the one found in largest amount within the muscles; and it is natural to suppose that creatine, which is capable of being converted in the laboratory without any great difficulty into urea and sarcosine, might be the immediate precursor of urea. It is, however, found that if creatine is injected into the blood or subcutaneously, or if it is taken with food, and thus absorbed into the blood, it does not become converted into urea, but is found in the urine as creatinine; and we cannot therefore suppose that the creatine of the muscles is absorbed by the blood, and carried by that fluid to the liver, and there converted into urea, since we find that creatine added to the blood does not become so converted.

Without ignoring the possibility that the creatine which is found in muscle may still be a preliminary stage in the transformation of proteid into urea, we must look for other products of nitrogenous metabolism passing from the muscles which, whether derived immediately from the proteid or indirectly from it through creatine, may be supposed to be the real precursors of urea. As a matter of fact, such products are found in the form of ammonia salts. It was noticed by Schöndorff, in the experiments already quoted, that in cases in which the blood of a fasting animal was sent through the limbs only of a well-

[1] Städeler and Frerichs, *Journ. f. prakt. Chem.*, Leipzig, 1858, Bd. lxxiii. S. 48.

nourished animal, there was an increase of ammonia salts in the blood. It is also a well-established fact that certain ammonia salts passed through the liver along with the blood become synthetised within the liver into urea. The actual form which such ammonia salts probably take is that of a combination with sarcolactic acid, which is also, as is well known, produced in muscle, and which is found, probably in combination with ammonia, in the blood generally.[1] These facts render it not improbable that the ultimate condition of the proteid which has been metabolised in muscle is lactate of ammonia (whether passing through the intermediate condition of creatine or not). This lactate of ammonia passing into the general circulation and being conveyed to the liver is there converted in mammals into urea, in birds into uric acid, in which form it is excreted by the kidneys. In conformity with this it was found by Marfori [2] that lactate of ammonia injected into the vein of a dog at the rate of 60 to 100 mgrms. per kilo. per hour, was wholly changed to urea.[3] The rest of the molecule, there can be very little doubt, is oxidised and got rid of in the form of carbon dioxide and water.

What intermediate changes may be gone through between the splitting of the proteid molecule into a nitrogenous and non-nitrogenous part, and the ultimate oxidation of the non-nitrogenous part into carbon dioxide and water, is a matter mainly of conjecture; but since we find within the muscular tissue, as an almost constant constituent, glycogen, it is conceivable that in part at least the split-off non-nitrogenous portion of the proteid molecule may first become converted into that substance, and possibly into grape-sugar, to be subsequently further split up and oxidised to form the ultimate products of oxidation.

That the proteid molecule can split up into a nitrogenous part and a part which is capable of being converted into carbohydrate, is shown very strikingly by the phenomena of diabetes, whether natural and of the severe form, or whether due to the administration of phloridzin or pancreatic extirpation. In these cases, even when the diet is exclusively proteid, or even when food is altogether withheld and the starving animal is compelled to live mainly upon the proteids of its own tissue, sugar becomes formed in great amount, and must be produced by the transformation of proteids. It is not unreasonable to suppose that this is merely an abnormally heightened form of the normal condition of things, and that under ordinary circumstances a similar transformation of the proteid molecule may go on in the muscles, for in phloridzin diabetes at any rate [4] the formation of sugar is independent of the liver.

With regard to the sulphur of the metamorphosed proteids, this

[1] Gaglio, *Arch. f. Physiol.*, Leipzig, 1886, S. 400. Gaglio showed not only that lactic acid is constantly present in the blood, but that its amount is increased in blood perfused through various "surviving" organs (kidneys, lungs). See also p. 159 of this volume. V. Frey also obtained an increase of lactic acid in blood which had been perfused through "surviving" muscle (*Arch. f. Physiol.*, Leipzig, 1885, S. 533).

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxi. S. 71.

[3] Minkowski found that in geese, after extirpation of the liver, the ammonia of the urine, which in normal geese amounts to from 9 to 18 per cent. of the total nitrogen, was increased to from 50 to 60 per cent. (*Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xxi. S. 41; and *ibid.*, 1893, Bd. xxxi. S. 214), whilst the uric acid almost disappeared. The ammonia was in the form of lactate, although in the normal animal there is no appreciable amount of lactic acid in the urine.

[4] In pancreatic diabetes, according to Marcuse (*Verhandl. d. physiol. Gesellsch. zu Berlin*, 1893–4, S. 98), this is not the case. Frogs deprived of liver, as well as pancreas, although they lived 3 to 5 days, showed no glycosuria.

undoubtedly is mainly transformed by oxidation into sulphate, for it is found that the sulphates of the urine go hand in hand with the amount of proteid metabolism which is proceeding (see also p. 630).

Nitrogenous metabolism in the liver.—That a very important part of the nitrogenous metabolism of the body occurs in the liver, has been insisted upon, and the experiments which have led to our knowledge on this matter have already been incidentally referred to.

Urea.—The evidence of the formation of urea in the liver was obtained by v. Schröder in a series of researches of remarkable interest and importance. Schröder[1] first determined that this substance was not formed in the kidneys, at least exclusively. He found that when the kidneys were extirpated in a dog, the amount of urea in the blood was increased in the next twenty-four hours to four times the normal quantity (from 0·05 per cent. to 0·2 per cent.). Nor was he able to obtain any increase of urea in blood passed through the kidney, even when such blood contained substances (*e.g.* carbonate of ammonia) which, by the liver, are capable of being synthetised into urea. This is the more striking, because, as we have seen, the kidneys are capable of performing such an important synthetic process as the formation of hippuric acid from benzoic acid and glycine (Bunge).

Schröder also showed that urea is not formed in the muscles. He found that blood containing carbonate of ammonia, when perfused through the hind-limbs of a dog, showed no increase of urea. On the other hand, blood similarly treated, and passed several times through the liver of a freshly-killed animal, was found to contain twice or three times the amount of urea which it had before passing through the organ. It was necessary for success in these experiments that the liver should be taken from a well-nourished animal. If removed from a fasting dog no urea was formed. A similar result, obtained by Schöndorff, has been already referred to (p. 903).

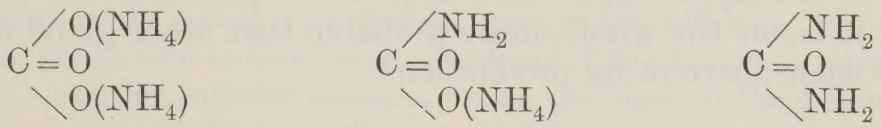
These experiments were repeated by Salomon[2] both upon sheep and dogs. They show conclusively that the liver is capable of forming urea from carbonate of ammonia. We have already seen that it is also capable of forming urea from blood containing the products of digestion. Other salts of ammonia besides carbonate are found to be effective; amongst others lactate and carbamate of ammonia, and also the amido-acids, such as leucine and glycine. On the other hand, in extensive disease of the liver, especially of rapid occurrence, and in experiments involving removal of the liver in mammals, combined with the establishment of a communication between the portal and the general venous system, so that there should be no stasis of blood in the capillaries of the intestinal circulation, ammonia salts are found to largely replace urea in the urine, such salts taking the form of lactates and of carbamates (p. 908). With partial extirpation of the liver,[3] and also with phosphorus poisoning, in which the liver cells undergo extensive degeneration, there is also a greater or less diminution of urea in the urine, and a corresponding increase of ammonia; as regeneration occurs, the urea becomes again gradually increased. The same has also been noted in extensive disease of the liver, especially when of rapid occurrence, but also in cases of

[1] See note 2, p. 902. [2] *Ibid.*

[3] Ponfick (in rabbit), *Virchow's Archiv*, Bde. cxviii. S. 225 ; cxix. S. 193, cxxxviii. Suppl. S. 81 ; v. Meister (in cat and dog), *Centralbl. f. allg. Path. u. path. Anat.*, Jena, 1891, Bd. ii.

cirrhosis; the ammonia is usually accompanied by lactic acid.[1] In acute yellow atrophy there is besides a considerable amount of leucine and tyrosine. When carbonate of ammonia is administered to mammals, the excretion of ammonia is not increased in the urine, but urea is formed in proportion to the amount of ammonium carbonate ingested.[2]

It is possible that, as Drechsel[3] supposes, carbamate of ammonia may be the immediate precursor of urea, the carbonate being first converted into carbamate and then into urea. By elimination of one molecule of water, carbonate of ammonia forms carbamate, and by elimination of a second molecule, urea.[4]



(carbonate of ammonia) (carbamate of ammonia) (urea)

When chloride of ammonia is administered with the food in herbivora (rabbits), the whole of the ingested ammonia appears as urea in the urine, but in carnivora and in man some of the ammonia is excreted in the urine.[5] The difference is due to the fact that the inorganic salts of the food of herbivora contain an excess of potassium carbonate. This takes the hydrochloric acid from the chloride of ammonia, and carbonate of ammonia is formed, which is readily converted into urea. But when there is an excess of proteid in the diet, sulphuric acid is formed by oxidation, and this combines with any bases present, so that the ammonia is not set free from the hydrochloric acid, and the chloride of ammonia passes unchanged (Bunge). In fasting dogs all the ammonia administered may be recovered from the urine.[6]

It is obvious that the formation by synthesis of the neutral substance urea from the alkaline salt, carbonate of ammonia, which is formed in the metabolism of proteid, is a protection from the deleterious effects which might otherwise ensue from too high an alkalinity of the circulating fluid and urine.

The presumption, therefore, undoubtedly is, that under ordinary circumstances the ultimate transformation of the products of nitrogenous metabolism takes place under the influence of the hepatic cells.[7]

[1] For references consult Bunge's "Lectures," pp. 327 and 345; also Neumeister, "Lehrbuch," S. 315.

[2] Hallervorden (with Schmiedeberg), *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1880, Bd. xii. S. 237; Feder and E. Voit, *Ztschr. f. Biol.*, Münehen, 1880, Bd. xvi. S. 177. Feder and Voit found that acetate of ammonia also forms urea. The same thing was determined for citrate of ammonia by Lohrer, with Buchheim (Diss., Dorpat, 1862). This is the reason why citrate of ammonia does not, like citrate of potash, make the urine alkaline. Both citrates are converted into carbonates in the body, but the carbonate of ammonia becomes transformed into the neutral urea, while the carbonate of potash passes as such into the urine.

[3] *Ber. d. k. sächs. Gesellsch. d. Wissensch.*, 1875, S. 171; *Journ. f. prakt. Chem.*, Leipzig, 1875, N.F., Bd. xii.; 1877, Bd. xvi.; 1880, Bd. xxii.; *Arch. f. Physiol.*, Leipzig, 1880, S. 550.

[4] Hoppe-Seyler (*Ber. d. deutsch. chem. Gesellsch.*, Berlin, 1874, Bd. vii. S. 34) and Salkowski (*Centralbl. f. d. med. Wissensch.*, Berlin, 1875, S. 913; and *Ztschr. f. physiol. Chem.*, Strassburg, 1877, Bd. i. S. 26) regard it as probable that cyanic acid may be the immediate precursor of urea, which is readily formed from cyanate of ammonia. But the evidence in favour of this supposition is by no means sufficient, whereas that in favour of ammonium carbonate (and carbamate) being the precursor, is very strong.

[5] v. Knieriem, *Ztschr. f. Biol.*, München, 1874, Bd. x. S. 263; Salkowski, *Ztschr. f. physiol. Chem.*, Strassburg, 1897, Bd. i. S. 1.

[6] Feder, *Ztschr. f. Biol.*, München, 1877, Bd. xiii. S. 256.

[7] Richet has shown that a urea-forming ferment can be precipitated by alcohol from aqueous extract of liver (*Compt. rend. Soc. de biol.*, Paris, 1894, p. 525).

It must not, however, be assumed that the whole of this ultimate metabolism occurs in the liver, for, as a matter of fact, after complete destruction of the liver by disease, or after its complete removal or destruction by operation, the urea of the urine does not wholly disappear, although in large measure replaced by ammonium salts (and under some circumstances by leucine and tyrosine). It is not certainly known in what other organs urea may be formed. The occurrence of a large amount of urea in the muscles of Elasmobranchs might seem to point to the muscles as the possible source of such urea.[1] But, on the other hand, as we have seen, v. Schröder was unable to obtain any evidence of the appearance of urea in blood perfused through muscles of dogs. It is, on the whole, more probable that other glandular organs may have some share in its production.[2]

The total removal or destruction of the liver in mammals has been rendered possible, by the discovery that it is feasible to establish a permanent communication between the portal vein and the inferior vena cava (Eck's fistula),[3] and thus, after tying the hepatic artery as well as the portal vein, to shunt the liver altogether out of the circulation; in fact, after such a fistula is established, the organ may be altogether removed. A large number of such operations have been made upon dogs by Hahn, Massen, Nencki, and J. Pawlow, and the results upon general, and especially nitrogenous metabolism, carefully recorded.[4] About one-third of the number in which the fistula was established recovered from the effects of the operation, but those in which the organ was completely removed only lived a few hours. Many of those with the Eck fistula refused food. These soon showed symptoms of convulsions, and eventually died. Those which fed well recovered weight, and showed no very obvious symptoms, unless given an excess of proteid food; this invariably brought on convulsions. The same result was produced by giving carbamic salts with the food (although these produce no such symptom in normal dogs). The urea was only slightly diminished in those with the Eck fistula only (but greatly so when the liver was completely removed); the uric acid excreted was at first greatly increased (four times), but afterwards became normal; the ammonium salts in the urine were increased, and were partly in the form of carbamate. Merely tying the hepatic artery in rabbits may also cause the appearance of ammonium lactate in the urine,[5] a result probably due to interference with its oxidation to carbonate of ammonia, and the synthesis of this to urea. The amount gradually diminishes, under these circumstances, as a collateral arterial circulation becomes established in the liver.

Nencki, Pawlow, and Zaleski[6] found that the portal blood of flesh-fed dogs contains three and a half times as much ammonia as the hepatic blood. Nevertheless the amount in the portal vein (which they calculated to have been 4·73 grms. in ten hours in a dog weighing 9·5 kilos.) was too small to

[1] The muscles of Elasmobranchs were found by v. Schröder (*Ztschr. f. physiol. Chem.*, Strassburg, 1890, Bd. xiv. S. 576) to contain 2 per cent. of urea; the blood, 2·6 per cent.; the liver, 1·36 per cent. The amount found in the muscles remained the same after removal of the liver. It is therefore evident that the conditions of nitrogenous metabolism are quite different in these animals from those met with in mammals.

[2] Cf. Münzer, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1894, Bd. xxxiii. S. 164; Kaufmann, *Arch. de physiol. norm. et path.*, Paris, 1894, p. 531.

[3] Eck, *Trav. Soc. d. natur. de St. Pétersbourg*, 1879, tome x.

[4] *Arch. de sc. biol.*, St. Pétersbourg, 1892, tome i. p. 401; *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxii. S. 161. See also Stern, *ibid.*, Bd. xix. S. 45; and v. Schröder, *ibid.*, S. 373.

[5] Zillessen, *Ztschr. f. physiol. Chem.*, Strassburg, 1891, Bd. xv. S. 387.

[6] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, Bd. xxxvii. S. 26.

account for the whole of the urea excreted by the kidneys; they consider, therefore, that it must be partly formed in other organs.

Uric acid.—In those animals in which the ultimate product of nitrogenous metabolism is uric acid instead of urea, it is probable that the primary changes which go on in the muscles result in the formation of similar substances (lactic acid and ammonia) as are formed as antecedents of urea, and that the secondary change to uric acid occurs in the liver. For, after extirpation of the liver in birds, these substances are found in the urine replacing uric acid.[1]

Minkowski[2] removed the liver in geese, taking advantage of the fact that in birds there exists a vein (Jacobson's vein) which effects a communication between the portal vein and the vena advehens of the kidney, so that when the portal vein is tied beyond this communication the blood of the mesenteric veins passes through the kidneys, and is not caused to stagnate as in mammals. In some animals he merely tied the portal vein, in others he completely removed the liver; these, however, only survived the operation a few hours (at most twenty). The uric acid of the urine sank as the result of this operation, so that from representing 60 to 70 per cent. of the total nitrogen of that secretion, as in normal animals, it represented only 3 to 6 per cent., the amount of ammonia in the form of lactate being correspondingly increased. The lactic acid found in the urine under these circumstances is the sarcolactic which is formed in muscular tissue; it is present in even larger proportions than ammonia, so that the urine is strongly acid. Altogether half the solids of the urine were formed of ammonium lactate, although in the normal animal none is present. No amido-acids were found in the urine, and no sulphates. The urea and creatine were unaltered in amount. In the blood, lactic acid was present and also some leucine and tyrosine. Urea injected into the stomach appeared as such in the urine, although in the normal goose it is converted into uric acid. The administration of glycine and asparagine caused a great increase in the ammonia of the urine. The same effect as extirpation—namely, the replacement of uric acid by lactate of ammonia—may be produced by merely tying the hepatic artery in birds,[3] a result which is probably due to the fact that the oxidations within the organ have been thereby so greatly interfered with, that the transformation of the lactate of ammonia into carbonate and the subsequent synthesis of uric acid has been prevented. That uric acid can be formed *in vitro* from lactic acid, ammonia, and carbonic acid, has been shown by Horbaczewski, who obtained uric acid by heating trichlorlactamide with urea (see p. 587).

We are, however, not justified in assuming that the uric acid which is found in the urine of mammals runs a parallel course in its formation with that taken in the formation of urea. For, in the first place, it is not in them, as in birds, necessarily increased in amount by the ingestion of proteid food, nor does it go hand in hand with the excretion of urea. And whereas in birds the ingestion of the amido-acids and of ammonia

[1] v. Schröder showed that uric acid is not formed in the kidneys in birds and snakes, but that after the removal of those organs it accumulates in the blood and tissues (*Arch. f. Physiol.*, Leipzig, 1880, Suppl. S. 113; *Beitr. z. Physiol. C. Ludwig z. s. 70 Geburtst.*, Leipzig, 1887, S. 89). v. Schröder also showed, in confirmation of an earlier observation of Meissner, that the liver of birds contains more uric acid than the blood. According to A. Garrod (*Proc. Roy. Soc. London*, 1893, vol. liii. p. 478), there is no uric acid normally in birds' blood, and this contains as much urea as that of a mammal. Garrod is of opinion that uric acid is produced in the kidneys by synthesis of urea and glycine. If the results of v. Schröder are correct, it is difficult to understand this conclusion.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xxi. S. 41.

[3] Minkowski, *ibid.*, 1893, Bd. xxxi. S. 214.

salts is followed by an increase of uric acid in the urine, such substances given with the food to mammals and man produce only an increase in the urea excreted. The same thing occurs even when uric acid itself is given to dogs, whereas the addition of urea to the food of birds produces an increase in the uric acid excreted.[1] There is in fact a fundamental difference in this respect between the proteid metabolism of mammals as compared with birds and reptiles, but the difference is in the later stage of such metabolism, which occurs in the liver, and not in the earlier stage, which occurs in the muscles. It has been noticed that in mammals the diet which most tends to produce an increase of uric acid is glandular substance and especially thymus gland, which contains a large amount of nucleo-proteid. It is not increased by a flesh diet, unless this includes nucleo-proteids; whereas in affections in which there is an increased formation (and presumably therefore also an increased destruction) of lymph cells (leucocytosis), a marked increase of uric acid has been noticed (*e.g.* in leukæmia).[2]

For a further discussion of this subject, see "Chemistry of the Urine," pp. 593–596.

Marès[3] found the uric acid of the urine to be increased during digestion; he ascribes this to the activity of glandular cells and increased metabolism of nucleo-proteids. In accordance with this view, he found pilocarpine to produce a similar increase. Horbaczewski[4] found the uric acid diminished in cases of cirrhosis of the liver, and concludes, therefore, that it is not formed in man in this organ. On the other hand, by digesting spleen pulp (which normally contains neither uric acid nor xanthin nor hypoxanthin) with arterial blood at 40° C., he obtained a considerable formation of uric acid, and the same when nuclein was used instead of spleen pulp.

Uric acid given to mammals (dogs) with their food does not increase the uric acid of the urine but the urea. In all probability it becomes transformed into urea in the liver. The uric acid which is found in the urine, and which has probably been formed from the nucleo-proteid of the food, or of degenerated cells of the tissues, may be supposed to have reached the kidneys without having passed through the liver.

If in a dog with an Eck's fistula the uric acid in the urine be estimated, and the hepatic artery then tied, it is found that the uric acid in the urine is largely increased. This may be explained by supposing that the uric acid formed from the products of disintegration of nucleo-proteids of cells, such as those of the spleen and lymphatic glands, has not been transformed into urea, as would be the case were it allowed to pass through the liver. Those products of disintegration are proteids, phosphates, and xanthin- (alloxuric) bases. The latter partly undergo further oxidation into uric acid, partly are eliminated directly by the kidneys as xanthin or hypoxanthin. They are increased in blood which has been perfused through almost all organs of

[1] Hypoxanthin given to birds also markedly increases the uric acid of the urine; this also occurs, however, when the liver is removed. It is therefore probably transformed by a process of simple oxidation in the tissues (v. Mach, with Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxiii. S. 139).

[2] Stadthagen, *Virchow's Archiv*, 1887, Bd. cix. S. 390, found no uric acid in the spleen or liver in cases of leukæmia, but abundance of xanthin and hypoxanthin. There was a great increase of uric acid in the urine, but this was not further increased by giving uric acid with the food.

[3] *Arch. slaves de biol.*, Paris, 1887, tome iii. p. 207.

[4] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1889, Bd. xcviii., Abth. 3, S. 301; *ibid.*, 1891, Bd. c. S. 78; *Arch. f. Physiol.*, Leipzig, 1893, S. 109. Cf. Marès, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, Bd. ci., Abth. 3, S. 12.

the body, but especially those with much lymphoid tissue.[1] In amphibia and fishes the oxidation to uric acid does not occur, and this substance is not found in the urine.[2] Part of the uric acid of the bird's urine appears to be produced in the same manner as in mammals, and does not disappear after extirpation of the liver.[3]

Influence of muscular activity on proteid metabolism.—As we have already insisted upon, the greater part of the metabolism of the body goes on in the muscles. This is the case both when at rest and in activity, but their metabolism is greatly increased during activity. This is sufficiently shown by the fact that a much larger amount of carbonic acid is given off from the body when the muscles are contracting actively than when in a condition of rest; and the chemical changes which can be shown to occur in the muscles as a result of contraction, such as the production of sarcolactic acid and of CO_2, as well as the disappearance of glycogen, must mean increased metabolism.

Although there is a general consensus of opinion that the CO_2 output of the body is largely increased as a result of muscular activity, the evidence that the CO_2 leaves the muscles as such is by no means conclusive. The observations which have been made upon this subject are of two kinds, namely, (*a*) the observation of the CO_2 given off by excised "surviving" frog's muscle, tetanised at intervals, as compared with the amount given off by corresponding muscle at rest; and (*b*) the observation of the amount of CO_2 in the venous blood of the muscles of mammals, taken during conditions of rest and of contraction of the muscles respectively. The best-known experiments of the first kind are those of L. Hermann,[4] in confirmation of the results of Matteuci[5] and Valentin,[6] who found that the CO_2 yielded by tetanised frog muscles was greater in amount than that yielded by resting muscles under like conditions. The difference, however, was greatly diminished by agitation of non-contracting muscles. A careful repetition of this experiment, conducted in 1893–4 in my laboratory, by L. Hill,[7] failed to show to the most careful analysis any appreciable difference in the CO_2 output of two such sets of muscles (contracting and resting). Similar experiments by Tissot[8] yielded results confirmatory of those of Hermann. Recently the question has been again investigated by Fletcher,[9] who employed a titration method for the estimation of the CO_2, and made use of the extremely accurate apparatus devised by Blackman[10] for estimating the gaseous exchanges in plants. Fletcher, both with skeletal and with cardiac muscle (tortoise), was able to obtain only the smallest possible difference of CO_2 output between rest and contraction, and he comes to the conclusion that the contrary results obtained by Hermann and others are due to the prolonged stimulation inducing the commencement of rigor mortis, a condition which is attended by a considerable output of CO_2. The other method of investigation, by the estimation of the CO_2 contained in the blood which has passed through muscular tissue, as compared with that entering it, was first undertaken by Ludwig and Sczelkow[11] upon muscles *in situ* in the

[1] Horbaczewski, *Monatsh. f. Chem.*, Wien, 1889, Bd. x. S. 624, and *loc. cit.*, *supra*.
[2] Nebelthau, *Ztschr. f Biol.*, München, 1889, Bd. xxv. S. 129.
[3] Minkowski, *loc. cit.*
[4] "Stoffwechsel der Muskeln," Berlin, 1867.
[5] *Ann. de chim. et phys.*, Paris, 1856, Sér. 3, tome 47.
[6] *Arch. f. physiol. Heilk.*, Stuttgart, 1857.
[7] Hitherto unpublished.
[8] *Arch. de physiol. norm. et path.*, Paris, 1894–5.
[9] Communication to the Physiological Society, May 1897, not yet published.
[10] *Phil. Trans.*, London, 1895, vol. clxxxvi. p. 485.
[11] *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1862, Bd. xlv.

living animal, and by Ludwig and Schmidt[1] upon separated and perfused muscles of the dog. Most of these experiments showed an increase of CO_2 during contraction, but in some there was no increase. Minot[2] (also with Ludwig), using serum for perfusion instead of blood, could find no relation between the CO_2 output and the contraction of the muscles. He came to the conclusion that CO_2 is not one of the disintegration products formed during contraction. Frey and Gruber,[3] using somewhat improved methods, have, however, obtained more distinct evidence of an increase of CO_2 during contraction; and a similar result was got by Chauveau and Kaufmann,[4] who investigated the amount of CO_2 in the blood passing to and from the levator labii inferioris of the horse when at rest, and when in natural activity during mastication. It must be stated, however, that the results obtained by perfusion of separated mammalian muscles are not altogether free from the objection raised by Fletcher regarding the excised surviving muscles of the frog, that prolonged excitation may tend to hasten the approach of rigor.

It is therefore not absolutely certain whether the CO_2, which is ultimately produced as a result of muscular activity, actually leaves the muscle as such, or in some other form, such as lactic acid, which is destined to be further oxidised elsewhere.[5]

The most interesting question in connection with the special metabolism of the muscles which remains to be considered, is the effect which their exercise produces upon the proteid metabolism of the body. It was the opinion of Liebig that the energy of muscular contraction was produced by the oxidation of muscular substance, and it would follow from this that the exercise of the muscles must tend, *ceteris paribus*, to increase the amount of nitrogen excreted in the urine. This doctrine of Liebig was accepted for many years by physiologists, but was, for a time at least, completely overthrown by the results of the famous experiment of Fick and Wislicenus,[6] known as the experiment of the ascent of the Faulhorn. It was shown by these observers that at least three times as much work was done during the ascent as could be accounted for by the oxidation of proteid, as estimated by the amount of nitrogen eliminated by them during and after the work.

The work, therefore, could only have been caused by the oxidation of non-proteid matter. Similar results were obtained by Parkes[7] and others in man, and by C. Voit in dogs.[8] This, combined with the fact that the CO_2 output of the body is increased in proportion to the amount of exercise, led to the view being widely adopted that the energy of the body is mainly, if not entirely, obtained by oxidation of non-proteid materials, and that the splitting and oxidation of proteid

[1] *Arb. a. d. physiol. Anst. zu Leipzig*, 1868.
[2] *Ibid.*, 1877.
[3] *Arch. f. Physiol.*, Leipzig, 1885, S. 519.
[4] *Compt. rend. Acad. d. sc.*, Paris, 1887.
[5] For other observations and statistics on the subject, see article "Chemistry of Respiration."
[6] *Vrtljschr. d. naturf. Gesellsch. in Zürich*, 1865, Bd. x. S. 317.
[7] *Proc. Roy. Soc. London*, 1872, vol. xx. p. 402.
[8] See Hermann's "Handbuch," Bd. vi. S. 187. The experiments of Parkes, Voit, and North (as well as those by Pavy, Austin Flint, and others, which cannot here be referred to in detail), were made upon men and dogs taking walking exercise. It has been determined by Zuntz and Katzenstein (in man and horse) that each kilogrammetre of ascent work is accompanied by a consumption of oxygen thirteen times that consumed in each metre of walking exercise (*Arch. f. Physiol.*, Leipzig, 1890, S. 367, *Verhandl. d. physiol. Gesellsch. zu Berlin*).

must contribute, under the ordinary circumstances of a mixed diet, but little to the production of muscular energy.

Many other experiments have been performed of a like nature, and leading practically to the same conclusion. A few, however, have given a result which has shown a somewhat increased amount of nitrogenous excretion,[1] but it will be found on an examination of these that in every case there has either been an excessive amount of work done, leading to probably an abnormal condition of metabolism,[2] or there has been taken in with the food an insufficient amount of non-proteid material to provide the necessary oxidation and energy for the work required to be done, *plus* the maintenance of body-temperature. Under such circumstances, it is clear that the proteid material of the food must be called upon for oxidation and the formation of energy, and we should then naturally expect an increased amount of urea in the urine.

Based upon experiments which come under this heading, Pflüger[3] and his pupils have shown a tendency of late years to return to the original doctrine of Liebig, and to throw over the view which has been accepted almost exclusively since the experiment of Fick and Wislicenus above referred to. Thus Argutinsky,[4] working with Pflüger, found, in repeating the experiment of Fick and Wislicenus in a somewhat modified form, that there was a marked increase (12 to 25 per cent.) of nitrogen excreted, if not so much during the actual performance of the work, at any rate during the two days succeeding it, and that from 75 to 100 per cent. of the total work done could be accounted for by oxidation of proteid; even with 100 grms. of sugar added to the diet, there was still an excess of N excreted sufficient to account for 25 per cent. of the work done. Similar experiments by Krummacher and others yielded a like result.[5] It has, however, been pointed out by I. Munk,[6] that the conditions of the experiments of Argutinsky are not the same as those of Fick and Wislicenus, in so far as the amount of food which was taken by Argutinsky and Krummacher had an insufficient caloric value to produce the required amount of energy, that of Argutinsky representing only 18 calories per kilo.; that of Krummacher only 28 calories per kilo., whereas a man *at rest* requires 32 calories per kilo.; as a natural result, a part of the proteids of the body was called upon for the production of the necessary energy. That, given a sufficient amount of proteid food, and an insufficient amount of non-proteid food, a large amount of muscular energy can be produced by oxidation of the proteid is no doubt true. Thus, a dog which was kept by Pflüger for some months upon lean meat, containing a very small amount of non-proteid

[1] Cf., for example, Austin Flint, *Journ. Anat. and Physiol.*, London, vol. xi. p. 109, and vol. xii. p. 91; Pavy, *Lancet*, London, 1876, vol. ii. Nos. 22–26; 1877, vol. i. No. 2; W. North, *Proc. Roy. Soc. London*, 1883, vol. xxxvi. p. 11.

[2] That such nitrogen increase is associated with abnormal conditions, appears from the experiments of Oppenheimer (*Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxii. S. 40 and Bd. xxiii. S. 446), of Zuntz and Schermberg (*Arch. f. Physiol.*, Leipzig, 1895, S. 378), and of Oddi and Tarulli (*Bull. d. r. Accad. med. di Roma*, tome xix. pp. 2 and 57).

[3] "Die Quelle der Muskelkraft," *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l. S. 98.

[4] *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xlvi. S. 552.

[5] *Ibid.*, 1890, Bd. xlvii. S. 454; see also Pflüger and Bohland, *ibid.*, 1885, Bd. xxxvi. S. 165; Bleibtreu and Bohland, *ibid.*, 1886, Bd. xxxviii. S. 1.

[6] *Arch. f. Physiol.*, Leipzig, 1890, S. 557 (*Verhandl. d. physiol. Gesellsch. zu Berlin*, 1889–90, No. 12). Cf. also Hirschfeld, *Virchow's Archiv*, 1890, Bd. cxxi. S. 501, who obtained a marked increase of N in the excreta when working on insufficient diet, but not when the diet, whether proteid or non-proteid, was sufficient. Further, Sondén and Tigerstedt, *Skandin. Arch. f. Physiol.*, Leipzig, 1895, Bd. vi. S. 181.

matter, was nevertheless able to perform a large amount of muscular work, metabolising at the same time a very considerable amount of proteid, the oxidation of which could have been the only source of the greater part of the energy. Whether, however, as Pflüger holds, the living tissue *prefers to employ* proteid, when it has sufficient offered to it, for the production of work, or whether, as is generally supposed, it uses up first the available non-proteid material for the production of energy, and only secondarily calls upon the proteid for the purpose of oxidation and energy production, is a matter which it is not at all easy to settle. That proteids enter largely into the diet of athletes is a fact which is of some importance in connection with the question. But although for short periods athletes are unquestionably capable of doing a very large amount of work, it must be remembered that their diet is by no means rigidly confined to proteid substances. It must also be borne in mind that there is a large class of labourers both in this, but more especially in other countries, who get through a much larger average amount of work per diem than is performed by athletes, and who, nevertheless, frequently have an amount of proteid in their diet the oxidation of which is altogether insufficient to account for the work done. The fact that a proteid diet has been selected for training purposes may be due, in the first place, to the more ready assimilation of proteid by the body; and, in the second place, to the fact that it is specially required in these cases, because during training it is important to encourage the building up of muscular tissue, and for this purpose proteid is necessary; not because the proteid of the diet is itself more readily oxidised and converted into energy by oxidation than the non-proteid materials.

But whether there may be produced under some circumstances as the result of muscular exercise an increase in the nitrogen of the egesta, it is certain that the most prominent effect upon the egesta of activity of the muscles is an increase in the amount of carbon dioxide,[1] such increase being either unaccompanied by, or altogether disproportionate to, any rise in the nitrogen egested. It must therefore arise from the oxidation of non-nitrogenous materials, *i.e.* fat and carbohydrates.

Cl. Bernard was of opinion that the grape-sugar which he had discovered in the liver and in the blood might by its oxidation in the tissues take an important part in the production both of heat and of mechanical energy. Seegen[2] is disposed to go much further than this, holding that muscular energy is obtained solely from the oxidation of dextrose brought to the muscles by the blood. He finds, as others have done, that sugar is never absent from the blood even after prolonged fasting, and that there is an excess of glucose in the hepatic blood, independent of the presence or absence of glycogen in the liver. He also finds in most cases a diminution in the percentage amount of sugar in the venous blood leaving a muscle, as compared with that in arterial blood. Some of Seegen's results are, however, paradoxical, nor have they received adequate confirmation, although a similar

[1] Cf. article "Chemistry of Respiration."

[2] "Die Zuckerbildung im Thierkörper," Berlin, 1890; and *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l., which volume also contains a criticism of Seegen's views by Pflüger. See also on this subject I. Munk, *Verhandl. d. physiol. Gesellsch. zu Berlin*, in the *Arch. f. Physiol.*, Leipzig, 1896, S. 372; Zuntz, *ibid.*, S. 538, and *Centralbl. f. Physiol.*, Leipzig u. Wien, 1896, S. 561; and Mosse, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 613. Other papers by Seegen relating to this subject will be found in the *Centralbl. f. Physiol.*, Leipzig u. Wien, during the last few years, and in the *Arch. f. Physiol.*, Leipzig, 1895 and 1896.

difference in the amount of sugar of the blood passing to and from an active muscle has been obtained by Chauveau and Kaufmann;[1] such differences as are found generally fall within the limits of experimental error. Chauveau[2] has further endeavoured to show that in dogs muscular work is not effected at all at the expense either of the proteids of the body or of the food; but, as I. Munk has pointed out, the results obtained cannot be accepted as conclusive.[3] That glycogen disappears both from the liver and from the muscles of dogs, and from the "surviving" excised muscles of the frog, concomitantly with the production of muscular work,[4] is held to be an argument in favour of this work being done at the expense of this carbohydrate. Under certain circumstances, however, the glycogen of the muscles may be caused to entirely disappear, although they are still capable of performing a large amount of work, which must, under these circumstances, be otherwise derived, however probable it may be that under normal circumstances the oxidation of dextrose or glycogen plays an important part in its production.

In support of the view that muscular energy may be largely derived from the oxidation of carbohydrate materials, it has been observed by Tiegel,[5] that the Japanese rickshaw runners consume rice in large quantities, and at frequent intervals, during their periods of work, whereas on off-days they live mainly on a flesh diet.[6]

Pflüger[7] kept a dog of 30 kilos. weight in equilibrium upon perfectly lean meat, containing a very large preponderance of proteids over non-proteids. When caused to pass from a condition of rest to hard work, it lost flesh if kept on the same diet, until it assumed a lower position of N-equilibrium, but maintained or even added to its weight if the amount of flesh was increased 500 grms. per diem; about 50 per cent. of the potential energy of the additional proteid appearing as work. If now, whilst in N-equilibrium on lean flesh, fat and carbohydrate were added to the diet, these were not utilised for the production of energy, but were stored as fat; hence, Pflüger argues, the living tissue prefers to use the proteid, and only takes non-proteid if insufficient proteid is offered to it. It should, however, be pointed out that Pflüger's dog was, with its purely proteid diet, in a condition of extreme emaciation,[8] and the cir-

[1] *Compt. rend. Acad. d. sc.*, Paris, 1887, tome civ. pp. 1126, 1352, and 1409. Similar results (disappearance of sugar from blood passing through active muscles) have been obtained by Morat and Dufourt (*Arch. de physiol. norm. et path.*, Paris, 1892, p. 327), who also found a certain disappearance *after* the work, which they suppose due to glycogen stored.

[2] With Contejean, *Compt. rend. Acad. d. sc.*, Paris, tome cxxii. pp. 429, 504.

[3] *Verhandl. d. physiol. Gesellsch. zu Berlin*, 8 Mai, in *Arch. f. Physiol.*, Leipzig, 1896.

[4] O. Nasse, *Arch. f. d. ges. Physiol.*, Bonn, 1869, Bd. ii. S. 97; also Weiss, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1876, Bd. lxiv. S. 288; Marcuse, *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxix. S. 425; Manché, *Ztschr. f. Biol.*, München, 1889, S. Bd. xxv. 164; and *ibid.*, 1877, Bd. xiv. S. 473.

[5] *Ibid.*, 1883, Bd. xxxi. S. 607.

[6] U. Mosso and Paoletti (*Atti d. Accad. d. Lincei*, Roma, 1893) and V. Harley (*Proc. Roy. Soc. London*, 1893, p. 480, and *Journ. Physiol.*, Cambridge and London, 1894, vol. xv. p. 97) using the ergograph of A. Mosso, found that they could perform a greater amount of voluntary muscular work when a large amount of cane-sugar was added to the diet. Experiments of this nature are, however, liable to a psychical error, and, as a matter of fact, experiments by Langemeyer (with Stokvis) made upon different persons failed to give similar results (see discussion in *Brit. Med. Journ.*, London, 1895, vol. ii. pp. 1280–1285).

[7] *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l. S. 98, and Bd. li. S. 317.

[8] It may be noted in this connection that the "Banting cure" for obesity depends upon the principle of selecting a diet not necessarily insufficient, but consisting mainly of lean meat. As in the case of Pflüger's dog, the tissues under these conditions use up the body-fat, which thus becomes gradually reduced in amount.

cumstance of the fat and liver cells seizing upon non-proteid materials and storing them as fat and glycogen in an animal in this condition, is not surprising, and is quite compatible with the view that in a normally nourished animal, where they are in excess, the non-proteids are the main energy producers.

The most probable view appears to be that muscle, like other cells, although it can only actually build up its bioplasm out of proteid, is nevertheless able to produce muscular energy by oxidation—perhaps occurring outside the actual molecules of the bioplasm, but under their direct influence—of any or all the organic foodstuffs,[1] and that this process is attended only by such small disintegration and loss of the proteid material of the bioplasm as is necessarily attendant upon its functional activity—a loss which is comparable to the wear and tear of the working parts of a machine as distinct from its consumption of fuel.

As a matter of fact, it has been shown by Zuntz,[2] that in a dog, abundantly fed on a mixed diet and caused to produce external work, the amount of extra proteid used during the period of work was less than one-twentieth part of the amount the oxidation of which would have been necessary to account for the work done. Moreover, in inanition it is the glycogen and fat of the body which is first drawn upon, and this both at rest and during work. When the same dog was made to work during fasting, the N-secretion rose only very slowly; the work was almost entirely done on the non-proteids of the body.

It may be remarked that muscular activity is always accompanied by a production of energy far in excess of that which is necessary for the performance of the external work done. Thus it was found by Hanriot and Richet[3] that when work was done there was seven times as much CO_2 produced as would have been accounted for by the oxidation necessary to perform the work. The additional energy appears of course as heat. On the other hand, it has been doubted whether there is any production of heat in the total absence of muscular activity.[4]

Hanriot and Richet found the CO_2 to increase in greater proportion than the oxygen absorbed, so that the respiratory quotient became larger. Severe muscular exercise is stated to increase both the phosphoric acid[5] and the sulphur of the urine;[6] the former more in proportion than the increase of N which may occur; the latter about in proportion to the increased N, and in the form of ordinary sulphates.[7] Along with the increase of phosphoric acid, there is also an increased excretion of lime, indicating, according to I. Munk, destruction of bony tissue.

Metabolism of Carbohydrate.

The formation of glycogen.—The carbohydrates of the food are mainly converted by digestion into maltose, which passes in the process of absorption and assimilation into dextrose, this being the only sugar

[1] Cf. Noël Paton, *Edin. Med. Journ.*, June 1895. Also *Rep. Lab. Roy. Coll. Phys.*, Edin., 1891, vol. iii.

[2] With Frentzel and Loeb, *Arch. f. Physiol.*, Leipzig, 1894, S. 541 (*Verhandl. d. physiol. Gesellsch. zu Berlin*). See also Speck, *ibid.*, 1895, S. 465.

[3] "Travaux du laboratoire de Ch. Richet," 1893, tome i.

[4] Cf. on this subject Speck, *Centralbl. f. d. med. Wissensch.*, Berlin, 1889; also article "Animal Heat," p. 840.

[5] Klug and Olsavsky, *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 21.

[6] Beck and Benedikt, *ibid.*, S. 27.

[7] I. Munk, *Verhandl. d. physiol. Gesellsch. zu Berlin*, 5th April 1895 (in *Arch. f. Physiol.*, Leipzig).

which is unmistakably found in the circulating fluids and in the tissues of the body. The path of absorption of carbohydrates is the same as that of proteids,[1] the absorbed dextrose being taken up by the blood, conveyed by the portal vein to the liver, and there stored. The portal blood taken during digestion is, in fact, the only blood in the body in which it can be conclusively shown that normally there is an excess of sugar. If taken in the intervals of digestion, it contains the same amount of sugar (one to two parts per thousand) as any other sample of blood.

The blood of the hepatic vein, on the other hand, although it is said to contain an excess of sugar in the intervals of absorption of foods containing carbohydrates (but *vide infra*, p. 923), does not, during the actual process of such absorption, contain nearly as much sugar as the blood of the portal vein; we must therefore assume that the sugar which is carried to the liver by the portal vein is arrested in that organ. As a matter of fact, it is found that the immediate result of the digestion and absorption of a meal containing much carbohydrate food is to promote a considerable accumulation of glycogen in the liver, and the same is found if in a fasting animal solution of dextrose is slowly injected into a vein of the mesentery,[2] or if dextrose is injected subcutaneously (in rabbits).[3] The same is even found if blood containing dextrose is perfused through the "surviving" liver of a dog.[4] The amount of glycogen in the liver (which would contain in man at most 150 grms. of this substance)[5] is not sufficient to account for the storage of the whole of the carbohydrate which is absorbed from a meal containing much starch or sugar. A part of the absorbed carbohydrate, when it is in excess, must therefore pass *through* the liver into the general circulation. Here it is apparently taken up by the muscles, for in a well-nourished animal, especially one nourished upon mixed food, the muscles may contain as much as 1 per cent. or even more of glycogen. Although this is not by any means as large a proportion as may be contained in the liver itself,[6] the muscles may collectively hold as much as is present in the liver.[7] Even, however, if we take into consideration the whole of the glycogen in the liver, that in the muscles, and that in other tissues in the body in which it might be stored, it will still be found that the whole of the carbohydrates of a meal which contains much of these substances is not represented in the body, either by the glycogen of the organs or by the sugar present in the

[1] See this Text-book, vol. i. pp. 432–436.

[2] Bernard, "Leçons de physiol. expér.," Paris, 1855.

[3] G. Lusk (with Voit), *Ztschr. f. Biol.*, München, 1892, S. 288. The ingestion or subcutaneous injection of lævulose will also cause a production of glycogen; galactose and lactose do not (C. Voit, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 245). Kausch and Solin (*Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxi. S. 398) obtained positive results with lactose and galactose. Cf. also Cremer, *ibid.*, 1893, Bd. xxix. S. 484; Haycraft, *Ztschr. f. physiol. Chem.*, Strassburg, 1894, Bd. xix. S. 141. Whether the lævulose is first converted into dextrose, and this into glycogen, or whether the glycogen is formed directly from lævulose, the ketone group of lævulose must in either case become converted into an aldehyde group (Neumeister, "Lehrbuch," Aufl. 2, S. 326). On the subject of the formation of glycogen from carbohydrates, see further, E. Voit, *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 551; C. Voit, *ibid.*, 1892, Bd. xxviii.

[4] Luchsinger, Inaug. Diss., Zurich, 1875.

[5] Bunge, "Lectures," p. 383.

[6] Pavy found in rabbits and dogs fed with a large amount of carbohydrate-containing food, that the amount of glycogen of the liver might rise as high as 17 per cent. ("Physiology of Carbohydrates," p. 116).

[7] Böhm, *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxiii. S. 51.

circulating fluid. The amount which is not accounted for may possibly pass into the constitution of the proteids and nucleo-proteids, and also of those albuminoids from which a carbohydrate material has been obtained on decomposition with acids, and it may be that the excess is in this way stored until required. In the embryo, glycogen is much more widely distributed and occurs in much larger proportion than after birth, especially in the developing muscles; at this time the liver may contain very little. It occurs also in considerable amount in the placenta.

The glycogen, both of the liver and of the muscles, gradually disappears in starvation, and first from the liver.[1] The disappearance is accelerated by muscular work,[2] and in warm-blooded animals by external cold;[3] it is probable, therefore, that the glycogen is used for the production of both work and heat. The rate at which it disappears in starvation varies greatly in different animals. Aldehoff found it in large quantity in the muscles of a horse which had fasted for nine days; in dogs it may be found after three weeks, and has been detected after thirty-five days' fasting;[4] in rabbits it has disappeared usually within a week. In frogs it accumulates in the liver towards the end of the summer, and gradually disappears during the winter; but even if they take no food, there is still some present at the end of the winter, but more in the muscles than in the liver. The same is the case with hibernating animals (Voit). On the other hand, if carbohydrates are given to animals deprived of their glycogen by starvation, this substance very rapidly reappears in the muscles and liver.[5]

The diminution of the glycogen of the muscles, concomitantly with their activity, has been already referred to in connection with muscular metabolism (p. 915). In the passage of excised muscles into the condition of rigor mortis there is a certain amount of disappearance, amounting, according to Werther,[6] to as much as 50 per cent. of the original amount, but far less than as the result of tetanising the muscles. In either case, what becomes of it is not clear; the sarcolactic acid which makes its appearance is not derived from it; the formation of the acid is not dependent upon the presence or absence of glycogen. If rigor is allowed to come on in the cold, the acid still appears, but there is no appreciable disappearance of glycogen.[7] On cutting the nerve proceeding to a muscle, the glycogen becomes increased in quantity.[8] The increase proceeds up to the fourth day. Section of the tendon of a muscle has a similar effect.[9]

According to the observations of Külz, glycogen begins to appear in the liver

[1] Aldehoff, *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 137; Hergenhahn, *ibid.*, 1890, Bd. xxvi. S. 225.

[2] Manché, *Ztschr. f. Biol.*, München, 1889, Bd. xxv. S. 163. The glycogen of muscles disappears after a period of tetanus, and also in frogs poisoned by strychnia, but not in the muscles of a leg the sciatic nerve of which has previously been cut.

[3] Külz, *Arch. f. d. ges. Physiol.*, Bonn, 1881, Bd. xxiv. S. 46. In cold-blooded animals external *warmth* produces the disappearance of glycogen from the liver (Langley).

[4] Quinquand, *Compt. rend. Soc. de biol.*, Paris, 1886, p. 285.

[5] For references, see Bunge, "Lectures," pp. 383–385. See also Langley, *Proc. Roy. Soc. London*, 1882, vol. xxxiv. p. 22 (histological observations); Quinquand, *Compt. rend. Soc. de biol.*, Paris, 1889, p. 285; Deweyre, *ibid.*, 1892, No. 19.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvi. S. 63.

[7] Böhm, *ibid.*, 1880, Bd. xxiii. S. 44.

[8] Bernard, *Compt. rend. Acad. d. sc.*, Paris, 1859, tome xlviii. p. 683.

[9] Boldt, Diss., Würzburg, 1893; Vay, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1894, Bd. xxxiv. S. 45.

of starved rabbits two to four hours after a meal containing carbohydrates, and disappears after five to eight hours' hard muscular work (dog). It is formed in the muscles of a frog which has been deprived of its liver;[1] and may be increased in muscular tissue by perfusing blood, to which grape-sugar has been added, through the vessels of the muscle.[2]

The formation of glycogen from other than carbohydrate material.—That glycogen can be formed in the entire absence of carbohydrate material from the food, is shown by the fact that animals which have been for a long time fed on lean meat, deprived as much as possible of carbohydrate, are found to have even considerable amounts of glycogen in their liver and muscles. Indeed, if an animal be allowed to fast for some days, and to perform also severe muscular work,—circumstances under which practically the whole of the glycogen can be made to disappear both from the liver and muscles,—on now administering proteid[3] or gelatin[4] food, altogether free from carbohydrates, glycogen will reappear both in the liver and in the muscles. Even without the administration of food, by the employment of narcotic drugs, such as chloral, which tend to diminish or arrest muscular activity, glycogen will reappear;[5] in this case it must be formed from the proteids of the body. The administration of fat without proteid does not cause such reappearance, nor does the addition of fat to the food, even in considerable excess, increase the amount of glycogen in the liver.[6] Arsenic poisoning causes a diminution in the glycogen both of the liver and of the muscles; probably by impairing the vitality of their bioplasm. On the other hand, the administration of glycerin promotes the storage of glycogen in the liver;[7] it acts, however, apparently rather by preventing the removal of the glycogen, than by becoming itself converted into that substance, or than by its becoming itself oxidized and thus acting as a glycogen sparer (Ransom). Thus it is found that with glycerin administration the sugar puncture is not able to produce glycosuria.

The administration of ammonium carbonate was also found by Röhmann[8] to promote the accumulation of glycogen in the liver, and this property is shared by many ammonium compounds,[9] but how they may act has not as yet

[1] Külz, *Arch. f. d. ges. Physiol.*, Bonn, 1881, Bd. xxiv. S. 64. This volume contains several other papers by Külz on the conditions of formation of glycogen.

[2] Külz, *Ztschr. f. Biol.*, München, 1891, Bd. xxvii. S. 237: there was, however, only an increase in three out of eleven experiments.

[3] Naunyn, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1875, Bd. iii. S. 94; v. Mering, *Arch. f. d. ges. Physiol.*, Bonn, 1876, Bd. xiv. S. 281; Külz, *Funfzigj. Doct.-Jubelf. d. . . . Carl Ludwig*, Marburg, 1890.

[4] Salomon, *Virchow's Archiv*, 1874, Bd. lxi. S. 352; Luchsinger, Inaug. Diss., Zurich, 1875; v. Mering, *loc. cit.*

[5] Zuntz u. Vogelius (*Arch. f. Physiol.*, Leipzig, 1893, S. 378, *Verhandl. d. physiol. Gesellsch. zu Berlin*) obtained a reappearance of glycogen on administering chloral to starved and strychnised rabbits.

[6] Chauveau has come to the conclusion that carbohydrate may be formed from fat in the animal body (*Compt. rend. Acad. d. sc.*, Paris, 1887, tome cxxii. p. 1098), and Seegen, "Die Zuckerbildung," also holds this view, but the evidence in its favour appears to be very insufficient.

[7] Weiss, *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1873, Bd. lxvii. S. 13; Eckhard, *loc. cit.*; Luchsinger, Inaug. Diss., Zurich, 1875; W. Ransom, *Journ. Physiol.*, Cambridge and London, 1887, vol. viii. p. 99; Schenck, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lvii. S. 569.

[8] *Arch. f. d. ges. Physiol.*, Bonn, 1886, Bd. xxxix. S. 21. Cf. also Külz (*Funfzigj. Doct.-Jubelf. d. . . . Carl Ludwig*, Marburg, 1890), who found that urea as well as ammonia salts increased the glycogen of the liver.

[9] Nebelthau, *Ztschr. f. Biol.*, München, 1892, Bd. xxviii. S. 138.

been determined. Bicarbonate of soda is stated by Dufourt to have the effect of increasing the amount of glycogen in the liver. Dufourt's experiments were made upon dogs on a flesh diet after a period of fasting.[1]

Glycogen becomes formed in the embryo chick in considerable amount, although there is very little glycogen or carbohydrate at all in the egg. Here also it must in all probability be formed from proteid. Glycogen can only be supposed to be produced from proteids in the animal body by a process of synthesis, preceded by a breaking down of the proteid molecule.[2] It is highly probable that dextrose is a stage in the course of such synthesis; and since dextrose is constantly found in the blood, even in prolonged inanition, it may well be inquired whether the carbohydrate of the body is invariably converted into glycogen, prior to being employed by the tissues for the production of energy. Under certain circumstances it appears clear that the synthesis of carbohydrate never passes beyond the stage of dextrose. Thus, in the diabetes produced by successive doses of phloridzin there may be no glycogen whatever in the liver and muscles, and yet within the proteid-fed and in the fasting animal large quantities of dextrose are formed and eliminated with the urine.

Phloridzin is a glucoside obtained from the root-bark of certain trees (apple and cherry), but it does not act by virtue of its glucose group, for the same action is got by the employment of the non-glucoside phloretin which is obtained from phloridzin. If injected under the skin, or taken into the alimentary canal, either phloridzin or phloretin produces within a very short time the appearance of sugar in the urine, and this appearance of sugar in the urine is accompanied by a diminution of the liver glycogen.[3] The glycogen in the liver does not, however, completely disappear as the result of a single dose of phloridzin; both in that organ and in the muscles a certain amount remains, but if a second dose of phloridzin is given, glycosuria is again produced, and by repeating the administration once or twice the glycogen can be completely removed from the liver. Each successive dose of phloridzin will, however, cause a fresh appearance of sugar in the urine even after complete removal of glycogen from the liver, which shows that, although part of the sugar which has appeared in consequence of the action of phloridzin may have been produced from the glycogen in the liver, a part must be produced in some other way. As by the employment of successive doses of this drug all the appreciable glycogen in the body can be got rid of,[4] it is almost certain that the sugar which then appears is derived from the metabolism of proteid; and this is rendered the more likely since it is

[1] *Arch. de méd. expér. et d'anat. path.*, Paris, 1890, tome ii. p. 424.

[2] Cf. Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1888, Bd. xlii. S. 144.

[3] v. Mering, *Verhandl. d. Cong. f. innere Med.*, Wiesbaden, 1887, S. 349; *Ztschr. f. klin. Med.*, Berlin, 1888, Bd. xiv. S. 405; 1889, Bd. xvi. S. 431. See also on phloridzin diabetes, Cremer and Ritter, *Ztschr. f. Biol.*, München, 1892, Bd. xviii. S. 459, and Bd. xix. S. 256; and Praussnitz, *ibid.* S. 168.

[4] Külz and Wright (*Ztschr. f. Biol.*, München, 1891, Bd. xxvii.) have shown that the glycogen is not so readily got rid of as v. Mering supposed, and that as a matter of fact there may still have been some glycogen left in the animals employed by v. Mering. These authors state that phloridzin does not produce glycosuria in frogs. It did, however, produce glycosuria in birds (v. Mering, *Verhandl. d. Cong. f. innere Med.*, Wiesbaden, 1887), in which pancreatic extirpation failed to cause glycosuria; it also increases the amount of sugar in the urine of animals suffering from pancreatic diabetes (Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxi. S. 148); and, further, Cremer has obtained phloridzin diabetes in frogs by taking special measures to ensure the action of the drug (*Ztschr. f. Biol.*, München, 1892–3, Bd. xxix. S. 175).

noticed that the amount of nitrogen in the urine goes hand in hand with the amount of sugar excreted.[1] Further, it is found that if the glycogen in the body be reduced as much as possible by a prolonged period of starvation, followed by excessive muscular action, such as is caused by a dose of strychnine, the administration of phloridzin will still cause glycosuria; much more sugar appearing under these circumstances in the urine than can be accounted for by any glycogen which might remain either in the liver or any other tissues of the body. It seems, therefore, clear that the sugar must have been derived from proteid; in this case the proteids of the body itself. It may further be mentioned that Pick[2] has found that if the liver be rendered functionless by injecting dilute sulphuric acid into the bile ducts, its glycogen disappears in twelve hours, but phloridzin still produces glycosuria, although other agents which usually cause glycosuria, such as carbon monoxide, fail to produce this effect. As with the glycosuria produced by phloridzin, so also with severe cases of natural diabetes in man, there appears to be no doubt that a direct formation of sugar from proteid may occur without any formation of glycogen. It may be supposed with some probability that such a direct formation of sugar (mainly by the liver, for phloridzin diabetes is produced in the absence of the liver),[3] but also by other organs;[4] and its passage into the blood may occur to some extent normally; that in fact a part of the carbohydrate produced from proteid may be at once passed into the blood in the form of dextrose, and a part further synthetised into glycogen and stored as such.[5] We might then explain phloridzin diabetes, and possibly certain severe cases of natural diabetes, by supposing that the further synthesis into glycogen is in some way interfered with, so that an excess of the carbohydrate formed is passed into the blood in the form of sugar.

It must, however, be stated that the production of the severest forms of diabetes above mentioned, and also that produced by removal of the pancreas (see p. 927) and by the sugar-puncture (see p. 926), is still exceedingly obscure. According to v. Mering and most other observers, there is a fundamental difference between the diabetes caused by phloridzin and that produced by pancreatic removal or sugar-puncture, in that in the former there is no excess of sugar in the blood,—in fact the amount may be less than normal,[6]—whereas in the two last-mentioned forms the

[1] v. Mering, *loc. cit.*, found the proportion of urea to sugar in phloridzin diabetes = 1 : 2, in cases of natural diabetes = 1 : 1. See also Moritz and Praussnitz, *Ztschr. f. Biol.*, München, 1891, Bd. xxvii. S. 81; Praussnitz, *loc. cit.*; Cremer and Ritter, *Ztschr. f. Biol.*, München, 1893, Bd. xxix. S. 256. v. Mering and Minkowski (*Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxvi.) found the proportion of sugar to nitrogen = 3 : 1 in pancreatic diabetes.

[2] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1894, Bd. xxxiii. S. 305.

[3] Thiel, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1887, Bd. xxiii.

[4] Cornevin (*Compt. rend. Acad. d. sc.*, Paris, 1895, tome cxvi. p. 263) has shown that phloridzin causes a marked increase in the amount of sugar eliminated in the milk.

[5] Seegen states that he obtained a formation of sugar (in excess of that produced by transformation of any glycogen present) in a mixture of chopped liver and arterial blood, to which peptone had been added, and even with the addition of fat in place of peptone. But his results have not been confirmed by other workers. Cf. Böhm u. Hoffmann, *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxiii.; Girard, *ibid.*, Bd. xvi. S. 294; Neumeister, *Ztschr. f. Biol.*, München, 1890, S. 346. The possibility of the formation of carbohydrate from fat in animals, although not experimentally proved, must not be ignored. For there is clear evidence that such a transformation may occur in germinating seeds of plants (Sächs, "Text-Book of Botany," transl. by Bennett and Thiselton Dyer, 1875, p. 638), and if plant bioplasm is capable of effecting the transformation, animal bioplasm might also be expected to have a similar power.

[6] No diminution but an increase in the amount of blood-sugar was found by Pavy to

percentage of sugar in the blood is greatly increased. This seems to point to the fact that phloridzin, besides any action it may have upon the metabolism of carbohydrate in the liver and muscles, increases the permeability of the kidney tubules to sugar, or causes the epithelium of the tubules to be more susceptible to the presence of sugar in the blood, so that the kidney removes sugar from that fluid more rapidly than under normal circumstances, and thus the percentage is even diminished below normal.[1] On the other hand, the diminution in the percentage caused by such removal, even if it were inappreciable to chemical methods of analysis, might be supposed to excite the sugar-producing tissues to increased activity, thus adding constantly more sugar to the blood, to be again removed by the kidneys, and so on in a vicious circle.

On the other hand, Levene[2] has given reasons for believing that the sugar in phloridzin diabetes may be produced in the kidneys, a view which was previously expressed by Uschinsky (quoted by Levene). Thus, after trying the renal blood vessels and then injecting phloridzin, there was no accumulation of sugar in the blood; indeed, the percentage of sugar in that fluid was, if anything, diminished. Minkowski[3] had previously failed to find an increase above the normal after ablation of the kidneys and injection of phloridzin, and Schabad[4] obtained analogous results after tying the ureters. Levene also finds that the amount of sugar in the kidneys is increased as the result of giving phloridzin, and that under the same circumstances there is rather more sugar in the blood of the renal vein than in that of the corresponding artery. He admits, however, the probability that it is formed in other organs as well as in the kidney. Minkowski[5] has put forward the suggestion that phloridzin becomes split up in the kidney into phloretin and sugar; the latter becoming eliminated, and the former combining again with sugar in the organism, and then again yielding this to the kidney, and so on.

Glycogenesis — Theory of Bernard.—As regards the fate of the carbohydrates of the food, there is no doubt that, whether they inevitably go through the stage of glycogen or not, they ultimately undergo oxidation into carbon dioxide, and removal in the form of this substance and water. The carbohydrate of the food directly increases the amount of carbon dioxide given off, and in proportion to the amount of such food taken. This elimination of carbon dioxide is not immediate, for most of the carbohydrate taken in is in the first instance stored, and only becomes oxidised gradually, as the needs of the organism demand. The view which has been most commonly held with regard to the method of transformation of the stored carbohydrate into the products of its oxidation, originated with Bernard. Having found that the blood of the hepatic vein constantly contains more sugar than the blood of the portal vein, except during the absorption of food, he concluded that the glycogen which he had discovered in the liver,[6] and which is no doubt the chief store of carbohydrate material in

occur in cats to which phloridzin had been administered ("Proc. Physiol. Soc.," Nov. 14, 1896, *Journ. Physiol.*, Cambridge and London, vol. xx.), and he therefore denies that diminished glycæmia is a feature of this form of diabetes.

[1] v. Mering, *loc. cit.*; Minkowski, "Untersuch. ü. d. Diabetes mellitus," Leipzig, 1893; Zuntz, *Arch. f. Physiol.*, Leipzig, 1895, S. 570.

[2] *Journ. Physiol.*, Cambridge and London, 1894–95, vol. xvii. p. 259.

[3] *Loc. cit.*

[4] *Vrach.*, St. Petersburg, 1892, No. 49, quoted from Minkowski.

[5] *Op. cit.*, p. 152.

[6] The fact that sugar is formed in the liver was discovered by Bernard in 1848 (*Compt. rend. Acad. d. sc.*, Paris, 1848, tome xxvii. pp. 249, 253, 514; "Nouvelle fonction du foie, etc.," Paris, 1853), but the substance (glycogen), from which it is produced was not found until 1857 (by Bernard, and also independently by Hensen). For a full list of Bernard's writings on this subject, see "L'œuvre de Claude Bernard," Paris, 1881.

the body, gives off such material into the blood in the form of dextrose. This dextrose is taken to the tissues and is used by them, becoming oxidised within them. Whether this oxidation occurs outside the actual bioplasm, or whether the dextrose which is taken to the bioplasm becomes first of all built up into its molecules and then split up and oxidised, and whether the products of its oxidation leave the muscles in their ultimate forms, are questions which we need not now consider. In either case the effect of such oxidation is to produce energy (in the form of heat and mechanical work).

This view of Bernard's has, on the whole, met with general favour among physiologists. Some there are, indeed, who have so far proceeded beyond Bernard, as to assert that the *whole energy of the body* is derived from the oxidation of carbohydrate. Such carbohydrate, which is taken to the tissues in the blood in the form of glucose, is assumed to be formed either from the stored carbohydrate of the liver, as Bernard supposed, or independently of this from proteid, or even from fatty materials in the liver cells, and being carried to the tissues to be taken up by them, oxidised within them, and thus become the immediate source of the energy of the body, whether this takes the form of heat or work. It is in fact assumed that the main result of metabolism within the body is the production in one part, and the destruction in another, of carbohydrate. Such a view has been chiefly contended for by Seegen [1] and Chauveau, who hold that even the proteid material of the food, at least its non-nitrogenous part, must ultimately become converted into carbohydrate before it can become oxidised in the tissues (see p. 914).

It is obvious that Bernard's theory is, in the main, dependent upon the circumstance that sugar is continually being passed from the liver into the hepatic blood, even during starvation, and this, in fact, has been directly affirmed by Bernard and others. Even in the fasting animal, sugar is found in the blood, except at the extreme end of an inanition period; and, according to the analyses of Seegen, it always occurs in larger amount in the hepatic blood, whatever be the nature of the food, whether proteid, fat, or carbohydrate, than in blood from any other source. This occurrence of dextrose in larger proportion in the hepatic blood than in the rest of the blood of the body, if it were completely and satisfactorily determined, would be a fact of fundamental importance, and would go very far to establish Bernard's theory upon a firm basis. But there are reasons for believing that such an excess of sugar as has been found by Seegen and other observers is not present under absolutely normal conditions. Seegen's experiments were made without anæsthetics, and it is a well-established fact that any operation upon an animal, which involves the production of pain, will immediately produce a transformation of the glycogen of the liver into sugar, and the appearance of an excess of sugar in the hepatic blood.[2] It is, in fact, admitted by Seegen

[1] "Die Zuckerbildung im Thierkörper," Berlin, 1890, S. 218, and numerous papers in the *Arch. f. d. ges. Physiol.*, Bonn, and in the *Centralbl. f. Physiol.*, Leipzig u. Wien.

[2] Seegen calculates that in man from 500 to 1000 grms. of dextrose may pass into the blood from the liver in twenty-four hours. But since his calculations are based upon experiments made upon animals in an abnormal condition so far as the carbohydrate metabolism is concerned, these numbers cannot be accepted. Cf. Abeles, *Med. Jahrb.*, Wien, 1886, S. 383; I. Munk, *Berl. klin. Wchnschr.*, 1890, S. 595; also Pflüger, *Arch. f. d. ges. Physiol.*, Bonn, 1891, Bd. l. S. 330, 396; Mosse, *ibid.*, 1896, Bd. lxiii. S. 613; Zuntz, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1896, S. 561. The blood is obtained either directly from one of the hepatic veins, or by passing a catheter up into the inferior cava, this vein being then blocked just below the reception of the hepatic veins by the inflation of an india-rubber bag; or a tube is passed down from the jugular vein through the right auricle into the inferior cava, and its bent end is made to enter one of the hepatic veins.

himself, that when the blood from the hepatic vein is collected under conditions of anæsthesia, the difference between the percentage amount of sugar in the hepatic blood and that in ordinary arterial blood becomes greatly diminished, if it does not altogether disappear.[1]

Bernard's views have been combated strenuously by Pavy,[2] whose method of experimentation is not open to the same objection as that of Seegen and others who have found a constant excess of sugar in the hepatic blood. Pavy takes blood from the animal immediately after it has been killed by a blow upon the head, and before there has been time for any change to have occurred in the liver, and he finds that blood which is collected under these circumstances from the inferior vena cava (including, therefore, the blood which has passed out from the liver) never shows any appreciable excess of reducing substances over blood obtained from other parts of the body. Results similar to those of Pavy have also been obtained, although under somewhat different conditions, by other observers.

We are therefore landed in this difficulty, as the result of the imperfection of our present methods, that we cannot be sure whether the blood of the hepatic vein does or does not, normally, contain an excess of sugar. If it does, we are bound to assume that sugar is being continually passed off from the liver into the general blood of the body, and since this sugar does not pass off by the urine, it can only be available for the nutrition of the tissues, and the production of energy by oxidation. If sugar does not pass from the liver into the blood, we should require to find some form in which the glycogen, which is undoubtedly stored up in the liver, is got rid of, and also to find some meaning for its presence there and in the muscles.

It has been suggested by Pavy[3] that such stored glycogen may become converted into fat. There is no doubt that carbohydrate food does become converted in the body into fat, and there are many instances of the formation of fat from carbohydrate material in plants; it is therefore not altogether wanting in probability, that the glycogen which is stored up in the liver cells and muscles may also become converted into fat. Such fat may be assumed to be gradually removed by the blood and carried to the different organs, and in them ultimately oxidised to carbonic acid and water.

Another supposition, which we have already considered, is that it becomes directly oxidised, and produces heat. As most of the oxidation of the body occurs in the muscles, and as the muscles retain their glycogen in starvation longer than the liver, although the latter organ contains normally a much larger proportion, it seems very probable that the glycogen passes from the liver to the muscles. This cannot be as glycogen, for glycogen is not present in blood plasma, and

[1] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1896–97, Bd. x. S. 497, 822.

[2] "The Physiology of the Carbohydrates," London, 1894. Here other papers by the same author are referred to.

[3] *Ibid.*, pp. 245 to 252. In connection with the question of sugar production by the liver, it may be mentioned that removal of this organ or cutting off its blood supply in rabbits (Bock and Hoffmann, "Exper. Studien ü. Diabetes," Berlin, 1874), dogs (Seegen, "Die Zuckerbildung," and Tanyl and v. Harley, *Arch. f. d. ges. Physiol.*, Bonn, 1895, Bd. lxi. S. 551), geese (Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1882, Bd. xx. S. 41), is followed by either disappearance or marked diminution of the sugar of the blood.

what little there is in the blood is in the white corpuscles—a property they share with most other protoplasmic structures. It is therefore natural to conclude, even if we cannot show the fact conclusively by analysis, that it passes from the liver to the muscles in the form of grape-sugar. The extra amount of sugar in the hepatic blood might be so small as easily to fall within the limits of experimental error, and yet sufficient to transport a very large amount of carbohydrate in the course of twenty-four hours.[1] Nor can it be said that we have any means of exactly estimating the amount of sugar in the blood at all. What has been estimated hitherto in the blood is not sugar alone, but substances which reduce cupric salts. That a part of these substances consists of glucose, is shown by the reaction with phenylhydrazine. But it must not be forgotten that there occur in the blood other substances which, although not glucose, also reduce metallic salts; nor can we say what proportion these hold to the glucose in the blood. Hence any mere determinations of the reducing substances do not give us a direct measure of the amount of glucose, and it is impossible to admit as proven any theory which is entirely built up upon observations of the amount of reduction yielded by the blood, on the assumption that such reduction is exclusively produced by glucose. If, therefore, we accept Bernard's theory, it must be understood that the evidence in its favour is mainly of an indirect character. There exists an analogy in the case of plants, in which the stored insoluble carbohydrate (starch) is conveyed from one part to another in the form of soluble sugars. And it must further be looked upon as a powerful argument in favour of Bernard's hypothesis, that under certain circumstances there is rapidly produced a very appreciable transformation of the liver glycogen into dextrose. This occurs as the result of stimulation of almost any sensory nerve, as the result of interference with the hepatic circulation,[2] and as the result of administration of many drugs. And it also occurs, as was found by Bernard early in his investigation of the subject, very rapidly after death, especially if the liver be kept at the body temperature. On the other hand, this transformation can be prevented by subjecting the liver, immediately after the animal is killed, to a sufficient amount of heat, as by throwing it in pieces into boiling water, or of cold, as by ice-cold salt solution,[3] or by a freezing mixture.[4] It has been held that this transformation, which occurs during the "survival" of the liver cells, is due to a continuance of such chemical processes as occur in the cells during life, and which lead to the change of their glycogen into sugar, just as the chemical changes which occur in muscle which is passing into rigor are generally similar to those produced during the

[1] Foster, "Text-Book of Physiology," 1889, pt. 2, 5th edition, p. 726.

[2] For these reasons conclusions should be drawn very cautiously from such experiments as those of the brothers Cavazzani (*Centralbl. f. Physiol.*, Leipzig u. Wien, 1894, Bd. viii. S. 33), who obtained disappearance of glycogen in the liver, and increase of sugar in the hepatic blood, on stimulation of the cœliac plexus. The same remark applies to the results obtained by Morat and Dufourt by excitation of the vagus (*Arch. de physiol. norm. et path.*, Paris, 1894, pp. 631 and 371).

[3] Dastre states that a temperature of 55° C. is sufficient to *destroy* the amylolytic action, and that prolonged exposure to ice-cold salt solution has the same effect. He argues from this that the action is not that of a ferment, but of cell protoplasm (*Arch. de physiol. norm. et path.*, Paris, 1888, p. 69). On the other hand, Nasse found that liver digested with chloroform water has a free amylolytic action, which must in that case be due to a ferment (*Rostocker Ztg.*, 1889, No. 105). See also Salkowski, *Centralbl. f. d. med. Wissensch.*, Berlin, 1889, No. 13).

[4] Pavy, "Physiology of Carbohydrates," p. 134.

activity of the muscular tissue; and accordingly anything, such as the sudden application of heat, able to instantly kill the liver cells stops such change.[1] On the other hand, it may also be that the transformation is caused by an amylolytic ferment, which is produced by the cells. This view was in fact held by Bernard,[2] but he afterwards supposed that the ferment was derived from the blood.[3]

It has been denied that such a ferment can be obtained from the liver, and it has therefore been contended that the transformation of glycogen into sugar must be produced by the direct metabolic action of the cell protoplasm. It has also been argued that, since the sugar which is produced by the digestive amylolytic ferments is maltose, and not dextrose, the production of dextrose in the surviving liver cannot be due to a ferment. Pavy, however, has shown that an active amylolytic ferment is obtainable from the alcohol hardened liver both in rabbits and cats, and that the sugar which is produced by it is closely similar to, if not identical with, that formed in the "surviving" organ.[4] A ferment converting glycogen into dextrose has also been obtained from the liver by Arthus and Huber,[5] and by Bial,[6] who states that it is identical with and probably derived from the diastatic ferment of blood and lymph.[7]

Puncture diabetes.—Bernard[8] also discovered the fact that certain lesions of the central nervous system, and especially a puncture in the region of the floor of the fourth ventricle, which corresponds, as we now know, very nearly to the position of the vasomotor centre, produces a condition of glycosuria; and that this is caused by a transformation of the glycogen of the liver into sugar, which is then taken up by the hepatic veins in so considerable a quantity, and increases so much the percentage of sugar in the blood, as to cause its excretion by the kidney. That this is the origin of the sugar in the so-called "puncture diabetes," is proved by the fact that, if precautions are taken to render the liver devoid of glycogen, as by a prolonged period of inanition,[9] with or without severe muscular activity, the glycosuria ordinarily resulting from puncture of the fourth ventricle does not appear, nor does it occur in frogs with the liver removed. It has been conjectured, with much probability, that

[1] Noël Paton found that if the liver substance be bruised up in a mortar with sand, so as to crush and thus destroy the liver cells, the change of glycogen into sugar does not occur (*Phil. Trans.*, London, 1894, vol. clxxxi. p. 233). But a repetition of his experiments by Pavy ("Epicriticism," London, 1895, p. 79) has not yielded the same results, and, since they were only few in number, they can hardly be accepted without further confirmation. Paton has, moreover, in later experiments, himself failed to verify his earlier results (*Journ. Physiol.*, Cambridge and London, 1897, vol. xxii. p. 121).

[2] "Leçons sur le diabète," Paris, 1877.

[3] *Compt. rend. Acad. d. sc.*, Paris, tome xli. p. 461.

[4] There seems to be little doubt that this sugar is mainly if not entirely dextrose (Seegen and Kratschmer, *Arch. f. d. ges. Physiol.*, Bonn, 1880, Bd. xxii. S. 214), but according to Chittenden and Lambert (*Stud. Lab. Physiol. Chem.*, New Haven, 1885) there is some maltose. Külz and Vogel also found a certain amount of both maltose and isomaltose in the fresh liver (*Centralbl. f. d. med. Wissensch.*, Berlin, 1894, S. 769). The remark that has been already made regarding the sugar found in blood applies to all these determinations of liver sugar, namely, that what is actually determined is the amount of reduction of cupric oxide, and that there may be, and undoubtedly are, other substances present besides sugar which effect this reduction.

[5] *Arch. de physiol. norm. et path.*, Paris, 1892, p. 651.

[6] *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. lv. S. 434.

[7] See article "Blood," p. 160.

[8] "Leçons sur la physiol. et la pathol. du système nerveux," Paris, 1858, tome i. p. 401. See also Eckhard, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giessen, 1869, Bd. iv. S. i. Kühne found the same thing to happen in frogs (Inaug. Diss., Göttingen, 1856).

[9] Luchsinger, *loc. cit.*

the condition of the liver which results from such puncture, and which tends to cause this transformation of the glycogen into sugar, is due to a disturbance of the hepatic circulation, and especially of the circulation in the hepatic artery, thus indirectly producing an alteration in the normal metabolism of the organ; but this cannot be considered as conclusively proved, and it may be due to a direct interference with the action of the nerves to the liver cells. Diabetes does not, however, occur on section of the splanchnic nerves alone.[1] But in all probability the vasomotor centre is stimulated by the puncture, for other forms of stimulation of the vasomotor centre also tend to produce a temporary diabetes, such as the prolonged stimulation[2] of most sensory or afferent nerves (*e.g.* the sciatic, the central end of the vagus,[3] and the depressor[4]), which are known so to influence the vasomotor centre as to produce constriction or dilatation of the arteries of the body generally. It is possible, therefore, that this may be the manner in which the effect is produced in the liver, and that the glycæmia is due to the diminution of the amount of oxygenated blood passing to the liver through the hepatic artery, causing an excitation of the liver cells, and such consequent alteration in their metabolic activity as ordinarily accompanies excitation.[5] Many drugs produce temporary diabetes, *e.g.* acids, such as phosphoric, lactic, and hydrochloric, also strychnine, curari, phosphorus, arsenic, carbon monoxide. Some of these may act by affecting the circulation, others by producing a dyspnœic condition of the liver cells, others again may be direct stimulants to the hepatic cells.[6]

It must be looked upon as a strong argument in favour of the glycogenetic theory of Bernard, that we find as a concomitant of the altered (increased?) activity of the hepatic cells, both after removal of the liver from the body, and after the diabetic puncture, such an increased production of sugar in the organ. It is certainly easier to explain the occurrence of puncture diabetes as an excess of the normal production of sugar in the liver, than as a phenomenon entirely *sui generis*.

Action of the pancreas on carbohydrate metabolism.—Until recently, it was not known that the pancreas had any more influence upon metabolism than other glands of the same type, such as the salivary glands. There had, however, been isolated instances recorded in which disease of the pancreas was accompanied by a condition of diabetes; but this, for the most part, was ascribed to the implication of the sympathetic ganglia, which are in anatomical relationship to the pancreas, and no special importance was attached to the pancreas in connection with the symptom.[7] It was, however, shown in 1889, by

[1] According to Kaufmann (*Compt. rend. Soc. de biol.*, Paris, 1894, p. 284), puncture diabetes is not produced, if the nerves both to the pancreas and liver are cut; but if one set only is severed, it is found to occur.

[2] Even that produced by sections, Külz, *Arch. f. d. ges. Physiol.*, Bonn, 1881, Bd. xxiv. S. 97. Here also will be found references to previous papers on the subject.

[3] Bernard, "Leçons sur le système nerveux," Paris, 1858, tome ii.; Eckhard, *Beitr. z. Anat. u. Physiol.* (*Eckhard*), Giesen, Bd. viii.

[4] Filehne, *Centralbl. f. d. med. Wissensch.*, Berlin, 1878, No. 18.

[5] Cf. Araki, *Ztschr. f. physiol. Chem.*, Strassburg, 1893, Bd. xvii.

[6] References to the literature of some of these substances will be given later; others will be found in Külz, *loc. cit.*, *supra*; and in Neumeister, "Lehrbuch," Aufl. 2, S. 328.

[7] Since the discovery by v. Mering and Minkowski of the effects of total removal of the pancreas, several cases of severe diabetes in man, associated with disease of that gland, have been recorded (G. Hoppe-Seyler, *Deutsches Arch. f. klin. Med.*, Leipzig, 1893, Bd. lii. S. 171; Buss, Diss., Göttingen, 1895). It must not be supposed, however, that this is at all common. In most cases of diabetes no affection of the pancreas can be substantiated.

von Mering and Minkowski,[1] that complete removal of the pancreas in the dog, cat, and pig,[2] is inevitably followed by a very severe form of diabetes, having the usual characters of that disease in man, namely, an enormous increase in the excretion of water, and the appearance in the urine, besides sugar, of aceto-acetic acid, acetone, and sometimes of oxy-butyric acid. That this condition is not in any way due to the abolition of the secretion of the gland, was further shown by the observation that it does not occur if the duct of Wirsung be tied, or if it and its branches be blocked by the injection of paraffin into them, and the gland left *in situ*, nor even if a certain proportion of the gland be left, its secretion being prevented from passing into the intestine; nor does it occur if a portion of the pancreas be detached from its normal position and transplanted elsewhere, either underneath the skin or in the peritoneal cavity,[3] and the remainder of the organ subsequently removed, although diabetes will appear in the severest form immediately after the removal of the transplanted portion from its subcutaneous situation.

The observations of v. Mering and Minkowski have been repeated and extended by Minkowski himself and by many other physiologists. The removal of the organ is less difficult than might be supposed, the chief precaution to take being to interfere as little as possible with the supply of blood to the duodenum. The complete removal is found invariably to be immediately followed by a considerable increase of sugar in the blood, where the amount of sugar may reach as high as 0·46 per cent., and its consequent appearance in the urine, in which the amount may rise to as much as 8 per cent. or more. In the increased amount in the blood pancreatic diabetes agrees with puncture diabetes, and differs from phloridzin diabetes, in which, as already stated, the amount of sugar in the blood is not increased, although there is a large increase of sugar in the urine. Concomitantly with this increase of sugar in the blood and its consequent appearance in the urine the glycogen of the liver disappears.[4] When no carbohydrate is given with the food, and even during prolonged fasting, the sugar continues to be eliminated in considerable quantity; and since, under these circum-

[1] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1889, Bd. xxvi.; see also Minkowski, *ibid.*, 1893, Bd. xxxi. S. 85. The experiments of v. Mering and Minkowski have been repeated by many observers, amongst whom may be mentioned especially Dominicis (*Gior. internaz. d. sc. med.*, Napoli, 1889), Hédon, Thiroloix, Gley, and Lépine (numerous papers during the last seven years in the *Compt. rend. Acad. d. sc.*, Paris; and in the *Compt. rend. Soc. de biol.*, Paris; in the *Arch. de physiol. norm. et path.*, Paris; and *Arch. de méd. expér. et d'anat. path.*, Paris); Vaughan Harley, *Journ. Anat. and Physiol.*, London, 1891, vol. xxvi.; *Journ. Physiol.*, Cambridge and London, 1891, vol. xii. p. 391; Caparelli (*Atti d. Accad. Gionenia di sc. nat. in Catania*, 1892, tome v.; Sandmeyer, *Ztschr. f. Biol.*, München, 1893, Bd. xxix. S. 86.

[2] The results in the rabbit were somewhat doubtful, and negative results were obtained in birds and in the frog. Aldehoff (*Ztschr. f. Biol.*, München, 1892, Bd. xxviii.), however, has obtained pancreatic glycosuria in the frog; as has also Marcuse (*Verhandl. d. physiol. Gesellsch. zu Berlin*, 1893–94, S. 98, in *Arch. f. Physiol.*, Leipzig), who states that it fails to occur if the liver be previously removed. This is also the case, according to Langendorff (*Arch. f. Physiol.*, Leipzig, 1887, S. 138), with the diabetes produced by strychnine and by puncture, but not with that produced by curari. Cf., however, Röhmann, *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, Bd. i. S. 122.

[3] Thiroloix ("Le diabète pancréatique," Paris, 1892) at first obtained a contrary result, but in later experiments (*Arch. de physiol. norm. et path.*, Paris, Oct. 1892) succeeded in confirming the original statement of v. Mering and Minkowski.

[4] According to Hédon (*Arch. de physiol. norm. et path.*, Paris, 1893), the *sugar* in the liver may nevertheless be increased in pancreatic diabetes. The administration of lævulose causes the reappearance of glycogen in the liver, although dextrose does not (Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxi.).

stances, its amount, as in the case of phloridzin glycosuria, rises and falls with the amount of nitrogen in the urine (see p. 921), it may be assumed that it is derived in these cases also from the splitting of proteids.

The exact amount of the pancreas which it is necessary to leave in order to prevent the occurrence of glycosuria cannot be exactly given, but a comparatively small amount is sufficient. If somewhat less than this minimum be removed, diabetes of a less severe type than that following complete removal may occur. There is, however, a tendency for it to become more severe in process of time, probably from a certain amount of atrophy occurring in the pancreatic tissue which has been left.[1]

Cause of pancreatic diabetes.—It appears probable that the pancreas exerts its influence upon carbohydrate metabolism, either by promoting the formation of glycogen in the liver from the dextrose taken to it by the portal blood, or by furthering the oxidation of dextrose in the tissues generally. In either case the effect would be the prevention of the accumulation of dextrose in the blood, so that the percentage of sugar in this fluid would be kept down to its normal, small amount. Whether this is brought about by the direct action of the organ upon dextrose which reaches it with the blood, or whether it acts indirectly in promoting the metabolism of dextrose by an internally secreted material, which passes out from the organ into the blood and tissues, is a question which it is impossible at present to give an answer to. Diabetes which results from removal of the pancreas, is not necessarily due to an increased glycogenesis from transformation of glycogen (although this is the cause of the glycosuria which first makes its appearance), for it will continue after the glycogen has completely disappeared from the liver and muscles. Moreover, the amount of sugar which is passed is altogether too great to be accounted for by the amount of glycogen present in the body. Nor is it due to a diminished consumption of sugar by the tissues.[2] It has been suggested that it is caused by the absence of the glycolytic ferment, which is described as being usually present in the blood. Lépine [3] has supposed that the pancreas forms such a glycolytic ferment, which effects the splitting of sugar prior to its oxidation in the tissues. But Minkowski [4] has shown that the blood of an animal deprived of its pancreas still possesses just as much power of glycolysis as a normal animal. Kausch,[5] who succeeded in producing diabetes in ducks and geese by pancreatic extirpation, also found that, after removal of the liver in the diabetic animal, moderate amounts of sugar were still consumed in the tissues. Pancreatic glycosuria diminishes or disappears during fever.[6]

The symptoms are not allayed by giving raw pancreas with the food, as those of thyroidectomy are by feeding with raw thyroid. Nor

[1] Cf. Hédon, *Compt. rend. Acad. d. sc.*, Paris, 1893, tome clvi. p. 649 ; and Thiroloix, *Arch. de physiol. norm. et path.*, Paris, 1892.

[2] Kaufmann, *Compt. rend. Acad. d. sc.*, Paris, 1894, tome cxviii. p. 656 ; *Arch. de physiol. norm. et path.*, Paris, 1896, p. 151.

[3] "Le ferment glycolytique et la pathogénie du diabète," Paris, 1891. Lépine's theory is supported by Vaughan Harley (*Brit. Med. Journ.*, London, 27th August 1892), and has been criticised by, amongst others, Spitzer (*Berl. klin. Wchnschr.*, 1894, S. 949).

[4] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxi.

[5] *Ibid.*, 1896, Bd. xxxvii. S. 274 ; and 1897, Bd. xxxix. S. 219.

[6] Kaufmann, *Compt. rend. Soc. de biol.*, Paris, 1896, p. 227. Fever was found by Poore to diminish sugar in natural diabetes (*Trans. Clin. Soc. London*, 1894).

are they due to any toxic substance accumulating in the blood (from which it might be supposed to be normally removed by the pancreas), as has been thought to be the case in the analogous instances of thyroid and suprarenal extirpation, for the blood of an animal rendered diabetic by pancreatic removal is not found to render a normal animal diabetic.

The facts clearly show that the diabetes which results from pancreatic extirpation is not the result of any interference with the sympathetic nerves in the neighbourhood of the organ, nor is it due to the arrest of the passage of the secretion of the gland into the intestine, but is exclusively the result of the removal of something belonging to the gland which acts in independence of its functions in connection with digestion. Since we find in the pancreas, if we compare its structure with similar glands such as the salivary, that the only important difference is the occurrence in the parenchyma of the pancreas of certain cell islands of an epithelium-like appearance richly supplied with blood vessels, and entirely unconnected with alveoli or gland ducts, it seems reasonable to suppose that the influence, whatever it may be, which the pancreas exerts upon carbohydrate metabolism, and which results in the excessive formation of sugar on its removal, is due to this particular tissue.[1]

That the salivary glands have no such influence upon metabolism as the pancreas was shown by Fehr,[2] and also conclusively by Minkowski,[3] who, after removal in dogs of all the salivary glands, including the orbital glands, found no appreciable effects either upon carbohydrate or any other form of metabolism to follow the removal. I have myself, in conjunction with Moore, repeated this experiment in a dog, removing in successive operations all the salivary glands upon both sides, leaving, however, the orbital glands. The animal remained in perfect health for several months, and no disturbances could be determined in either carbohydrate or proteid metabolism.[4]

Metabolism of Fat.

Is the fat of the body directly derived from the fat of the food?—That the fat of the body should be derived from the fat of the food seems at first sight extremely probable. But, on consideration, it will appear that before it is laid down as the fat of the tissues it would probably undergo a change. For the fat of different animals has by no means the same composition. Whereas some, such as the dog and man, have a large amount of olein in their adipose tissue, and consequently their fat has a comparatively low melting point, others, such as the sheep, have a large proportion of stearin, and the fat of such animals has a relatively high melting point.

Now, if a dog or a man is fed upon sheep's flesh and fat, the fat which is laid up in the body has not a different composition from that which it ordinarily possesses. That is to say, a man living upon mutton will have his body-fat, not of the consistency of mutton suet, but of the ordinary consistency of the fat of the human body, having a melting point far lower and containing a much larger amount of olein in its composition.

If, therefore, the fat of the food is laid down as the fat of the body, it must undergo important modifications. It is possible to suppose that only such portions of the fat of the food as would make fat of the

[1] Schäfer, "On Internal Secretions," *Brit. Med. Journ.*, London, August 1895.
[2] Inaug. Diss., Giessen, 1862 (quoted from Minkowski).
[3] *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1893, Bd. xxxi. S. 141.
[4] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. xiii.

composition normal to the particular species of animal, are laid down directly, and that other portions, such as the excess of stearin which occurs in mutton fat, become broken down completely, and either directly oxidised, or the products of their decomposition again built up to form the normal fat. It has indeed been conclusively proved that the fat of the food may be to a certain extent laid down unaltered in the body-fat. Dogs which have been starved for a considerable time, so that practically the whole of the body-fat has become removed, will, if fed upon an excess of mutton fat and sufficient proteid, lay down a body-fat of a melting point and composition very similar to mutton-fat. This shows that at least a portion of the fat introduced with the food has been, for a time at any rate, laid down directly as body-fat.[1]

It has been further shown that dogs to which there has been administered, along with their food, forms of fat which do not ordinarily occur in the animal economy, will lay down a certain amount of this along with their body-fat. This has been determined for spermaceti, linseed oil, and rape oil.[2] That in pigs the fat of the body may also be derived from the fat of the food, was shown in some of the experiments by Lawes and Gilbert.[3]

Formation of fats from fatty acids.—The question of the form in which fats are absorbed has been already considered in a previous article dealing with that subject, and it has there been shown that the fats of the food are in large part not absorbed in the form of fat, but in that of fatty acid, into which and glycerin they are broken up by the fat-splitting ferment of the pancreatic juice; and that they undergo a subsequent synthesis into fat by combination with glycerin in the columnar epithelial cells of the small intestine.

That such synthesis is possible even in the absence of glycerin given with the food, is shown by the experiments of I. Munk, who found that when a dog was fed upon fatty acids in place of the fats of its ordinary food, just as much fat was absorbed into the chyle and was laid down in the body as if it had been fed with the complete fat. The columnar epithelial cells become filled with fat globules, as after a meal containing actual fats; and the synthesis of fatty acid and glycerin to form fat must therefore have occurred in these cells, which must themselves have produced, in some way which is not understood, the glycerin necessary for the synthesis.[4]

Are fats formed from carbohydrate?—This is a question of great practical importance, seeing that carbohydrate foods are the cheapest forms of nutriment, and that the fattening of animals is an important branch of agricultural industry. The experience of all rearers of animals for market points to the fact that carbohydrates do produce fat. Sheep and oxen fed purely upon grass, which contains hardly any fat and but little proteid in proportion to the carbohydrate present, lay on a large amount of fat, and the artificial foods which are used for fattening purposes

[1] Lebedeff, *Centralbl. f. d. med. Wissensch.*, Berlin, 1882, S. 129; *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 149; *Arch. f. d. ges. Physiol.*, Bonn, 1883, Bd. xxxi. S. 11; I. Munk, *Arch. f. Physiol.*, Leipzig, 1883, S. 273 (*Verhandl. d. physiol. Gesellsch. zu Berlin*); *Virchow's Archiv*, 1884, Bd. xcv. S. 407.

[2] Radziewski, *Virchow's Archiv*, 1868, Bd. xliii. S. 286; Lebedeff, *loc. cit.*; I. Munk, *loc. cit.* See also Minkowski, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xxi. S. 373, and I. Munk and Rosenstein, *Virchow's Archiv*, 1891, Bd. cxxii. S. 230, for evidence that foreign fats pass into the chyle.

[3] See note 2 on next page.

[4] For further details regarding these and similar experiments, see article on "Fat Absorption," p. 443.

also for the most part contain, in addition to a certain amount of proteid, a large proportion of carbohydrate. In spite, however, of this almost universal experience, it has been held by C. Voit[1] that the carbohydrates of the food are not directly transformed into the fat of the body, but that they only act in promoting the fattening of animals by sparing the oxidation of proteid, so that the non-nitrogenous portion of the proteid molecule may become transformed into fat. It has been, in fact, altogether denied by Voit that the carbohydrates themselves can be transformed by the animal economy into fat, in spite of the well-established fact that in plants there frequently occurs, especially in the ripening of many seeds, a considerable transformation of carbohydrate material into fat. The question was, however, brought to the test of direct experiment by Lawes and Gilbert.[2] These observers took two pigs of the same litter, killed one as a control, and determined the total amount of fat in its body, and kept another one alive for some weeks, feeding it with proteid and an excess of carbohydrate food, and determining the exact amount of proteid in such food, then killed it, and determined the total amount of fat in its body. They found that the amount of fat which had been added on during the time could not be accounted for by supposing it to be derived from the proteids of the food, since there was not sufficient proteid in the food during the period of the experiment to account for more than two-thirds of the fat which had been formed, even supposing the whole of its non-nitrogenous moiety to have been transformed into fat. Therefore a part at least of the fat formed must have been derived from the carbohydrate in the food.

This experiment has since been repeated by subsequent observers on different animals,[3] and always with the same result, so that it may be taken as conclusively proved that the carbohydrate of the food may be converted into fat. The same fact may be shown by balance experiments, in which, with nitrogenous equilibrium, there is carbon disappearance in the egesta, showing that carbon is stored in the body in quantity more than to be accounted for by the carbon of the proteid metabolised; such laid up carbon must be mainly stored as fat.[4] Nor is this formation of fat from carbohydrate by any means a unique phenomenon in the organic world. As we have seen, it occurs in plants, in the seeds of which fat is deposited at the expense of sugar or starch; and in the process of fermentation of sugar, acids of the

[1] Hermann's "Handbuch," 1882, Bd. vi. S. 251 to 260.

[2] The very numerous original experiments by these observers, which were begun in 1847 in the private experimental agricultural station at Rothamstead, are described in the following amongst other publications:—*Rep. Brit. Ass. Adv. Sc.*, London, 1852 and 1854; *Journ. Roy. Agric. Soc. Eng.*, London, 1849, 1851, 1852, 1853, 1855, and 1860; *Phil. Trans.*, London, 1859; *Scient. Proc. Roy. Dublin Soc.*, 1864; *London, Edinburgh, and Dublin Phil. Mag.*, London, 1866; *Journ. Anat. and Physiol.*, London, 1877. An excellent historical and critical account of the part taken by the various foodstuffs in the metabolic processes of the animal economy is given by the same authors in *Journ. Roy. Agric. Soc. Eng.*, London, 1895, Ser. 3, vol. vi. pp. 47–141.

[3] Soxhlet, *Ztschr. d. Landw. Vereins in Bayern*, 1881; B. Schultze (geese), *Landw. Jahrb.*, 1882; Tscherwinsky, *Landw. Versuchst.*, Berlin, 1883, Bd. xxix. S. 317. (These are quoted from Neumeister, "Lehrbuch," S. 368.) See also Chaniewski (geese), *Ztschr. f. Biol.*, München, 1884, Bd. ii. S. 179; C. Voit, *Sitzungsb. d. k.-bayer. Akad. d. Wissensch. zu München*, 1885, S. 288; Meissl, Strohmer, and v. Lorenz (pig), *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 63; I. Munk (dog), *Virchow's Archiv*, 1886, Bd. ci. S. 91; Rubner (dog), *Ztschr. f. Biol.*, München, 1886, Bd. xxii. S. 272.

[4] Meissl and Strohmer, *Monatsh. f. Chem.*, Wien, 1883, Bd. iv. S. 801; *Sitzungsb. d. k. Akad. d. Wissensch.*, Wien, 1883, Bd. lxxxviii.; and *Ztschr. f. Biol.*, München, 1886, *loc. cit.*; Rubner, *loc. cit.*

fatty series are formed. And although it is not easy at first sight to understand, from a chemical point of view, how carbohydrate molecules are transformed into fatty molecules, we are not obliged to assume direct transformation, for it may well be that the carbohydrates are broken down into comparatively simple compounds, and that these are built up again by the organism into fat.

The observations of Hanriot, with Richet,[1] furnish indirect evidence of the transformation of carbohydrate into fat. These observers found that, with the administration of carbohydrate food, there is a greatly increased output of carbon dioxide without a corresponding increase of oxygen intake. This fact may be explained, according to Hanriot, by a transformation of carbohydrate into fat,[2] in conformity with such an equation as the following:—

$$13(C_6H_{12}O_6) = \underset{\text{(oleo-stearo-palmitin)}}{C_{55}H_{104}O_6} + 23(CO_2) + 26(H_2O)$$

Are fats formed from the proteids of the food?—This is a question which was for many years held to have been settled by the experiments of Pettenkofer and Voit, and subsequently of Voit.[3] These observers found that if a dog is kept in a respiration chamber, and fed entirely on lean meat, all the ingesta and egesta of the body being carefully determined and analysed, a comparison of the results shows clearly that in many cases carbon of the proteid is retained within the body, and is presumably in the form of fat, the amount of fat and carbohydrate in the food being altogether too small to suppose that the carbon laid by could have been derived from anything but the proteids of the food. Moreover, proteid food increases the amount of fat in the milk of suckling animals, and a bitch fed upon lean meat may produce much more fat in her milk than can be accounted for by the fat and carbohydrates of the food—produces, indeed, milk especially rich in fat, when fed exclusively on lean meat.[4]

In confirmation of observations of this kind have been adduced the statements that the milk of suckling animals and of nursing women is richer in cream in proportion to the amount of proteid taken in the diet; that fat becomes formed in large amount by the larvæ of blow-flies, which are fed upon defibrinated blood, containing only very small quantities of non-proteid organic material;[5] that in the ripening of cheese there is a diminishing amount of proteid, and an increasing amount of fat;[6] and that in the formation of adipocere from flesh, there is found a diminished amount of proteids, and an increased amount of fatty acids.[7] The formation of fat in the liver and tissues of a starving

[1] *Compt. rend. Acad. d. sc.*, Paris, 1892, tome cxiv. p. 371.

[2] Cf. also Gautier, *ibid.*, p. 374.

[3] *Ann. d. Chem. u. Pharm.*, 1862, Suppl. Bd. S. 52 and 361; *Ztschr. f. Biol.*, München, 1869, Bd. v.; also 1870 and 1871, Bde. vi. and vii.; art. "Ernährung," in Hermann's "Handbuch," Bd. vi. S. 249.

[4] Ssubotin, *Virchow's Archiv*, 1886, Bd. xxxv. S. 561; and *Centralbl. f. d. med. Wissensch.*, Berlin, 1866, S. 337; Kemmerich, *ibid.*, S. 467. Both Ssubotin and Kemmerich worked with Pflüger. See also Voit, *Ztschr. f. Biol.*, München, 1869, Bd. v. S. 137.

[5] Fr. Hofmann, *Ztschr. f. Biol.*, München, 1872, Bd. viii. S. 159.

[6] See on the changes accompanying the ripening of cheese, Sieber, *Journ. f. prakt. Chem.*, Leipzig, 1880, N. F., Bd. xxi. S. 203; Jacobsthal, *Arch. f. d. ges. Physiol.*, Bonn, 1893, Bd. liv. S. 484.

[7] Lehmann (*Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1885, S. 19) obtained an increase of fatty acids to the extent of 3·7 per cent. in meat kept in running water for some months. E. Voit (*München. med. Wchnschr.*, 1888, S. 518) got an increase of 2 per cent. when it was kept in milk of lime, thus excluding fungi.

animal poisoned by phosphorus[1] also affords strong presumption of the conversion of proteid into fat. The fact that in the deposition of fat in embryonic adipose tissue the fatty globules are preceded by albuminous granules may also be given as evidence in the same direction.

So important did the extent of such formation of fat from proteid appear to Voit, that he endeavoured, as already stated, to account for the fattening qualities of carbohydrate food by supposing that it mainly acts by sparing the oxidation of the proteids (and fats), thus allowing a larger amount of these to be transformed into body-fat.[2] In support of his views, he pointed out that if the proteid molecule is supposed to be split up, and a portion be removed in combination with the nitrogen as urea, the carbon, oxygen, and hydrogen which remain are not very different from the proportion of these elements which would be necessary for the formation of fat. It was indeed calculated by Henneberg[3] that 51·4 per cent. of proteid taken as food might, under the most favourable circumstances, be supposed to be converted into fat. Rubner, however, has shown that this estimate is too high. He calculates that the utmost amount which could be converted into fat would be about 46·9 per cent.

The view that the fat of the body is *exclusively derived* from the proteid of the food is, however, no longer held by any physiologists, and Voit has himself shown that it must in some circumstances be derived from carbohydrate.[4] The above view cannot, indeed, be held, if we accept, as we undoubtedly must, the conclusions to be drawn from experiments like those of Lawes and Gilbert. These experiments do not by any means exclude the formation of fat from proteid, but do exclude the possibility of its being formed entirely from proteid, and not from any other article of diet. That a certain amount of proteid is necessary to be added to the diet of a fattening or suckling animal is a matter of everyday experience; but it does not seem to be necessary that this proteid should be greatly in excess of that which is necessary to make up for the proteid lost from the tissues, or, in the case of the suckling animal, for that also which appears as caseinogen in the milk. If, however, the amount of proteid in the food is too much decreased, there is more call upon the carbohydrates and fats of the food for the immediate production of energy, and as a result there will be less of these to be transformed into fat.

[1] Storch, abstr. in *Deutsches Arch. f. klin. Med.*, Leipzig, 1867, Bd. ii. S. 264; Bauer, *Ztschr. f. Biol.*, München, 1871, Bd. vii. S. 63; *ibid.*, 1878, Bd. xiv. S. 527; Caseneuve, *Rev. mens. de méd. et chir.*, Paris, 1880, tome iv. pp. 265 and 444; Stolnikoff, *Arch. f. Physiol.*, Leipzig, 1887, Suppl. S. 1. Bauer found in a fasting dog, to which phosphorus had been administered, as much as 42 per cent. of fat in the muscles, and 30 in the dry liver substance, as against 16·7 in the muscles and 10 per cent. in the liver of control dogs. The nitrogen excreted is at the same time greatly increased, this also pointing to increased metabolism of proteid, while there is at the same time a diminished excretion of carbon dioxide, and correspondingly less oxygen taken in. A similar formation of fat from proteid in phosphorus poisoning has been shown by Leo (*Ztschr. f. physiol. Chem.*, Strassburg, 1885, Bd. ix. S. 483) to occur in frogs. On the other hand, Lebedeff (*Arch. f. d. ges. Physiol.*, Bonn, 1883, Bd. xxxi. S. 11) found in dogs which had previously been fed with linseed oil, that the fat in the liver cells, which was formed after administration of phosphorus, had the same characters as that which had been laid on in the adipose tissue; thus indicating a transference of fat to the liver rather than its formation there from proteid. Stolnikow found in frogs, after extirpation of the "fat-body," that the liver became enlarged, and fat accumulated in it under conditions of both carbohydrate and proteid nutriment, even without the addition of phosphorus to the diet.

[2] For further details of the evidence in favour of this view, see Voit, in Hermann's "Handbuch," Bd. vi. S. 243–251.

[3] Quoted by Voit, Art. in Hermann's "Handbuch," S. 250.

[4] *Biol. Centralbl.*, Erlangen, 1886–7, Bd. vi. S. 243.

That fat is formed from proteid, although not in the exclusive form in which Voit at one time was disposed to assert, has been almost universally accepted by physiologists; but this view has been strenuously attacked of late by Pflüger[1] who has criticised the conclusions drawn by Voit from his experiments of feeding dogs upon meat, and has shown that in all probability the meat employed contained sufficient fat to account for the fat laid on in the body without supposing this to have been derived from proteid. In a dog kept by himself and fed upon a large quantity of meat containing the least possible fat, no fat whatever appeared to be laid on; but what was originally present disappeared, so that the dog, although muscular and capable of performing severe work, was reduced to a condition of extreme leanness. Pflüger is therefore disposed to deny altogether the formation of fat in the animal body from proteid,[2] and considers that its sources are to be looked for exclusively in the fats and carbohydrates of the food.[3]

In this it would appear probable that Pflüger has gone as much to the one extreme as Voit originally went to the other. It is unquestionable that certain forms of bioplasm are capable of transforming proteid into fat (as in the instances cited on p. 933). This is, in fact, admitted by Pflüger, who, however, contends that we have no right to assume that other forms of bioplasm, such as that of the cells of the higher animals, possess the same power. He is disposed to regard the change as due in all the cases cited to the action of bacteria and fungi, such as would undoubtedly be present in ripening cheese, in putrefying blood, in putrefying flesh, and the like. But it has been shown that in flesh kept in milk of lime, and therefore under conditions unfavourable to the growth of bacteria, fatty acids are still found to a small extent, at the expense of the proteid; and the production of fatty degeneration in the cells of starved animals, to which phosphorus has been administered, is strong evidence in favour of their possessing such a power of forming fat from proteid; these, taken in conjunction with the numerous other instances which have been cited, appear to indicate that this power of forming fat from proteid is a general property of bioplasm.

As regards the ultimate fate of fat, there seems to be no doubt that it becomes oxidised into carbon dioxide and water, thus producing energy which may take the form of either heat or work, and that this oxidation takes place mainly in the muscular tissue.

Action of the liver in connection with the metabolism of fats.—Very little is known on this question beyond the fact that, under certain circumstances, there is a considerable accumulation of fat in the liver cells. This has been held by Pavy[4] to indicate the correctness of his view, that fat may be formed both in the liver and elsewhere by the direct transformation of glycogen. But it has not been shown that the glycogen and fat have any vicarious relation to one another; indeed, the contrary was found to be the case by Langley[5] and by Noël Paton.[6] Nevertheless, Paton's experiments show a marked increase in the fatty

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1892, Bd. li. S. 229; *ibid.*, 1892, Bd. lii. S. 1 and 239.

[2] Kumagawa and Kaneda, *Mitth. a. d. med. Fac. d. k.-jap. Univ.*, Tokio, 1894, Bd. iii. (abstr. in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1895, S. 721), were also unable to obtain evidence of fat formation in dogs fed upon food consisting almost exclusively of proteid.

[3] For a reply to Pflüger's criticisms, see E. Voit, *München. med. Wchnschr.*, 1892, S. 460, and *Ztschr. f. Biol.*, München, 1896, Bd. xxxii. S. 139; also Cremer, *ibid.*, 1897, S. 811. Pflüger's answer to these is in *Arch. f. d. ges. Physiol.*, Bonn, 1897, B. lxviii. S. 176. See also on this subject, I. Munk, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lviii. S. 309; also *Berl. klin. Wchnschr.*, 1889, No. 9.

[4] "Physiology of Carbohydrates," p. 258.

[5] *Proc. Roy. Soc. London*, 1882, vol. xxxiv. p. 20; and 1885, vol. xxxix. p. 234.

[6] *Journ. Physiol.*, Cambridge and London, 1896, vol. xix. p. 167. Langley's statements are founded upon microscopical observations (in the frog); Paton's, upon chemical evidence.

acids of the liver of the rabbit at a period after food when the glycogen is diminishing, and he concludes that they may have been formed from the glycogen.[1] Langley[2] has shown that in frogs there is a gradual accumulation of fat in the liver, chiefly in the outer zones of the cells, during the winter months, a time during which the glycogen is also gradually increasing; and, further, that both the liver fat and glycogen tend to diminish on warming the animals in winter. The glycogen becomes rapidly used up in the spring, and this is also the case with the fat. Paton found (in pigeons) that the liver fat did not appreciably diminish as the result of a four days' fast. Taken by itself, the presence of fat in the hepatic cells merely indicates that these cells may act as a temporary storehouse for fat. Whether such fat has been formed by them from carbohydrate or proteid, or whether it is directly derived from the fat of the food, and is in process of transformation in the liver cells into a fat more intimately allied to the fat of the body, are points which have not yet been determined, but the latter supposition appears the more probable; for excess of fat in the food is certainly largely stored in the liver cells.[3] And it has been noticed by Lebedeff,[4] and this observation is confirmed by Paton,[5] that the fats of the liver contain less oleic acid, and have a higher melting point, than those of the body generally. Moreover, as Hofmann showed,[6] there is a higher proportion of free fatty acids in the liver, pointing, according to Nasse, to an active metabolism of fats in that organ.[7] Lebedeff[8] found in geese which had been fed for six weeks upon peas, which are rich in proteid but contain very little fat, that the liver, although containing much lecithin, had no fat; and that the fat of the omentum was also only present in small amount. A large amount of proteid in the diet of rabbits and kittens was found by Paton not to lead to any accumulation of fat in the liver.[9]

[1] According to Paton, nearly one-half of the fatty acids of the liver are in combination with lecithin. See also Heffter, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1891, Bd. xxviii. S. 97; and Stolnikow, *Arch. f. Physiol.*, Leipzig, 1887, Suppl. Heft, S. 1.

[2] *Loc. cit.*

[3] Paton, *loc. cit.*, p. 202.

[4] *Ztschr. f. physiol. Chem.*, Strassburg, 1882, Bd. vi. S. 139.

[5] *Loc. cit.*, p. 179.

[6] *Beitr. z. Physiol. C. Ludwig z. s.* 70, *Geburtst.*, Leipzig, S. 134.

[7] *Biol. Centralbl.*, Erlangen, 1886–7, Bd. vi. S. 235.

[8] *Loc. cit.*

[9] *Loc. cit.*, p. 211.

THE INFLUENCE OF THE DUCTLESS GLANDS UPON METABOLISM—INTERNAL SECRETIONS.[1]

By E. A. Schäfer.

Contents :—Introductory, p. 937—The Thyroid Gland, p. 938—The Pituitary Body, p. 945—The Suprarenal Capsules, p. 948—The Spleen, p. 959.

Certain organs of the body have a special influence upon some of the metabolic processes of the body. Thus the liver fulfils important special functions in connection with the metabolism of carbohydrates and proteids, and of those organic compounds which contain iron; the pancreas has an obscure but absolutely essential function in connection with carbohydrate metabolism; and removal of a large portion of the kidneys has been shown by Bradford to produce a large increase in the proteid waste of the tissues.[2] It is also a matter of common knowledge that removal of the ovaries or testicles may produce profound modifications in the development of other organs, and in the general nutrition of the body. In the case of the pancreas (and perhaps in that of the kidney) it is by no means improbable that the gland yields to the blood some material which influences the carbohydrate (and nitrogenous) metabolism of other tissues. In the case of the generative glands this is perhaps less probable: it is on the whole more likely that these react upon the rest of the organism through the nervous system. Numerous observations have of late been published, commencing with those of Brown-Séquard, which have seemed to indicate that extracts of or the expressed juices of these glands produce, when injected hypodermically, beneficial effects upon the nervous and muscular systems, but it is not clear that this property is not shared by other organs rich in nuclein. Watery extracts or decoctions of the generative glands have very much the same action, if injected into a vein, as have extracts of other glands. In addition to the above instances, there are certain organs of a glandular structure, but destitute of ducts, which yield to the blood substances, which are in some cases at least absolutely essential to the due nutrition of the body, so that the results of the complete removal of these organs is inevitably fatal. These substances are no doubt formed by a process of secretion, but since they do not find their way to any free surface by means of a duct, but

[1] The substance of this chapter was originally given in the form of an address to the British Medical Association, and was published in the *British Medical Journal* for August 10, 1895. For the purposes of this book it has been carefully edited and many additions have been made to it ; references to literature have also been appended.

[2] *Proc. Roy. Soc. London*, 1892. vol. li. These researches of Bradford have already been noticed in a previous article (p. 656). See also Meyer, *Arch. de physiol. norm. et path.*, Paris, 1894, p. 179.

presumably reach the blood by means of the lymphatics or blood vessels of the organ, they have been termed "internal secretions."[1]

The Internal Secretion of the Thyroid Gland.

The first internal secretion which may be considered is that of the thyroid gland. That the thyroid is a secreting gland no one who studies its structure and its mode of development can well doubt; except that it is unprovided in the adult state with a duct, it has all the features of structure of secreting glands. It is formed of alveoli which are lined by epithelial cells; and although these cells have not been observed to exhibit changes characteristic of secretory activity so marked as those which have been noticed under like circumstances in the cells of ordinary glands,[2] we can observe the secreted material within the vesicles of the thyroid in the form of the substance known as "colloid." Various attempts have been made to isolate the active principle of the secretion; these are referred to in a previous article.[3] According to Drechsel,[4] there is probably more than one active substance, and the secretion may subserve more than one essential function.[5]

The gland is extremely vascular and very richly provided with nerves, and both blood vessels and nerves come into very close relationship with the secreting epithelium. The glandular structure of the thyroid is more obvious in young than in old animals, and as age advances, as has been shown by Hale White[6] and others, the organ undergoes a gradual process of degeneration, so that in advanced age its normal glandular structure can only with difficulty be recognised.

Effects of ablation and disease.—As long ago as 1856, Schiff[7] found that extirpation of the thyroid in dogs is invariably followed by a fatal result. This observation, important as it now seems, fell for many years into oblivion, and it was not until clinical observations had again pointed to the importance of the gland that Schiff's experiments were remembered. It has indeed long been recognised that extensive disease of the thyroid, such as occurs in advanced forms of goitre, are accompanied by a swollen appearance of the integument, giving a misshapen aspect to the features and to the extremities, and leading to an idiotic or semi-idiotic condition, which is known as cretinism. In 1873, Gull[8] described a series of symptoms, and especially a condition of the integumental connective tissue, similar to that which is met with in cretins, the name "myxœdema" being subsequently given to it, because it was believed to be an œdematous condition characterised by the presence of a large amount of mucin. That there is an excess of mucin over that in ordinary connective tissue has been shown

[1] In one sense all the tissues and organs of the body form internal secretions, for they all pass into the blood materials which have been formed as products of their metabolism.

[2] Hürthle, *Arch. f. d. ges. Physiol.*, Bonn, 1894, Bd. lvi. S. 1; Anderson, *Arch. f. Anat. u. Entwcklngsgesch.*, Leipzig, 1894, S. 177; Schmidt, *Arch. f. mikr. Anat.*, Bonn, 1896, Bd. xlvii. S. 181; Galeotti, *ibid.*, Bd. xlviii. S. 305.

[3] Halliburton, "Chemistry of the Tissues and Organs," p. 88.

[4] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1895, S. 705.

[5] Cf. also Notkin, *Virchow's Archiv*, 1896, Suppl. Bd. cxliv. S. 224; Hutchison, *Journ. Physiol.*, Cambridge and London, 1896, vol. xx. p. 474.

[6] *Lancet*, London, 1888, vol. i. p. 521.

[7] "Untersuch. ü. die Zuckerbildung," Würzburg, 1859.

[8] *Trans. Clin. Soc. London*, October 24, 1873 (in vol. vii., 1874).

by the analyses of Halliburton;[1] but not to the extent believed when the term myxœdema was applied to this condition. Nor is the condition really one of œdema, but rather of hyperplasia of the connective tissue, which becomes altered, assuming a more embryonic character, hence its richness in mucin (see note 1, p. 942). The connection of myxœdema with affections of the thyroid was first recognised by Ord[2] in 1878—an observation which has since been abundantly confirmed. In 1882, J. L. Reverdin[3] described the symptoms which follow complete removal of the thyroid body for goitre in man, and recognised these symptoms as identical with those of the disease which had been described under the name of myxœdema; he accordingly termed the collection of symptoms "operative myxœdema."[4] The results of Reverdin were speedily followed by those of Kocher, who described, in a large number of cases, similar symptoms as following entire removal of the thyroid in man.[5] Kocher pointed out that the effects are most marked in young subjects, and that they may not occur at all or be little manifest as age advances. These observations of Reverdin and Kocher led to a renewal of his former experiments by Schiff,[6] who in 1884 published the results of sixty thyroidectomies upon dogs, in all of which the result was speedily fatal,[7] the operation being quickly followed by the supervention of symptoms—tremors, spasms, and convulsions—which seemed to point to a serious derangement in the nutrition of the central nervous system.[8] Schiff also discovered the fact that the symptoms are prevented by a previous graft of a portion of the gland beneath the skin or into the peritoneal cavity.

Dogs do not show the swollen condition of the connective tissues which is a characteristic feature after thyroidectomy in man; this appears to be due to the fact that in them a fatal result usually occurs too rapidly to allow of the development of the so-called "myxœdema." They are liable, amongst other symptoms, to a form of conjunctivitis

[1] *Trans. Clin. Soc. London*, 1888, vol. xxi. Suppl.; *Journ. Path. and Bacteriol.*, Edin. and London, 1892.

[2] *Med.-Chir. Trans.*, London, 1878. See also Hadden, *Brain*, London, 1883, p. 193, and S. Mackenzie, *Trans. Clin. Soc. London*, 1888 (Report of Committee on Myxœdema), for an account of the symptoms of myxœdema in the human subject. For a very full bibliography of observations on the thyroid and its connection with myxœdema, see Ord, in Allbutt's "System of Medicine," 1897, vol. iv.

[3] *Rev. méd. de la Suisse Rom.*, Genève, 1882, p. 539; 1883, Nos. 4 to 6; 1887, pp. 275, 328.

[4] Termed also "cachexia strumipriva" and "cachexia thyreopriva."

[5] *Arch. f. klin. Chir.*, Berlin, 1883, Bd. xxix. S. 254.

[6] *Rev. méd. de la Suisse Rom.*, Genève, Feb. and Aug. 1884, Bd. xviii. S. 25.

[7] Schiff's dogs lived at longest fourteen days, when both lobes were simultaneously removed; if the removal was effected in two sittings, at a certain interval apart, the advent of the characteristic symptoms was delayed or altogether averted.

[8] These experiments of Schiff have been confirmed by many subsequent observers, but the literature of the subject is enormous, and only a few papers can here be mentioned—Wagner, *Wien. med. Bl.*, 1884, S. 25 and 30; Sanguirico and Canalis, *Arch. per le sc. med.*, Torino, 1884, tome viii.; Horsley, *Proc. Roy. Soc. London*, 1884 and 1886, and *Brit. Med. Journ.*, London, 1885, vol. i. p. 3; 1892, vol. i. p. 267; also *Festschr. Rudolf Virchow*, Berlin, 1891; Fuhr, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1886, Bd. xxi.; 1889, Bd. xxv.; Rogowitsch, *Centralbl. f. d. med. Wissensch.*, Berlin, 1886, S. 530, and *Arch. de physiol. norm. et path.*, Paris, 1888, p. 419; Albertoni and Tizzoni, *Centralbl. f. d. med. Wissensch.*, Berlin, 1885, S. 419; Hoffa, *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1887, S. 104; Ewald, *Berl. klin. Wchnschr.*, 1887, 1889, and 1895; v. Eiselsberg, "Ueber Tetanie im Anschluss an Kropfexstirpationen," *Wien. klin. Wchnschr.*, 1890; Gley, *Arch. de physiol. norm. et path.*, Paris, 1892, and subsequent volumes; Cristiani, *ibid.*, 1893; Langhans, *Virchow's Archiv*, 1892, Bd. cxxviii. S. 400; Domenicis, *Wien. med. Wchnschr.*, 1895, S. 1620; G. Rouxeau, *Arch. de physiol. norm. et path.*, Paris, 1897, tome ix. p. 136.

with leucocytic infiltration of the cornea.[1] Horsley was the first to operate upon monkeys. He found that these animals survive the removal of the thyroid much longer than do dogs, and that in them, as in man, a "myxœdematous" condition gradually supervenes. They also pass into a condition which is unmistakably similar to cretinism, besides which, as in dogs, they are subject to the supervention of muscular tremors. These, however, may be greatly abated, or their onset delayed, if the animals are kept in a very warm atmosphere.

It was further found by Allara[2] and by Ewald[3] that no results are obtainable by thyroidectomy in birds (although it is fatal to reptiles), and most observers found the same to be the case with rodents and herbivora generally.[4] It is a noteworthy fact that in aged dogs thyroidectomy does not produce the normal symptoms. In man also it is found that the supervention of operative myxœdema is less frequent as age advances.[5] The absence of the result in birds has never yet been satisfactorily explained; but various attempts have been made to explain the frequent absence of result in herbivora.[6] It may be, either that a portion of the gland has been left behind, or that the animals were not kept under observation for a sufficient time (Horsley), or that there existed in the particular case in question accessory thyroids and parathyroids, separated from the main body and not removed in the operation. This is the explanation which is given by Gley of the negative results which usually attend thyroidectomy in rabbits. Gley states that if in these animals care be taken to remove the accessory structures as well, the usual symptoms supervene and are very rapidly fatal.[7] Moreover, he finds that if in young dogs all the parathyroids are removed, while the main body of the thyroid is left intact, the symptoms which have been regarded as characteristic of complete thyroid removal nevertheless supervene, an observation which, if confirmed, shows that it is these structures which are physiologically the more important part of the organ.[8]

The symptoms which follow thyroidectomy are of two classes—nervous and metabolic, although we are not able to say that the nervous symptoms are not produced by metabolic changes in the tissues of the nervous system; nor is it certain that the metabolic changes in

[1] Gley and Rothon-Duvigneaud, *Arch. de physiol. norm. et path.*, Paris, 1894, p. 101.

[2] *Sperimentale*, Firenze, 1885, p. 281.

[3] Ewald and Rockwell, *Arch. f. d. ges. Physiol.*, Bonn, 1890, Bd. xlvii. S. 160.

[4] Sanguirico and Orecchia (abstract in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1887, Bd. i. S. 587).

[5] Bourneville and Bricon (*Arch. de neurol.*, Paris, 1886) have shown that the liability to constitutional symptoms usually ceases at about the thirtieth year.

[6] According to Briesacher (*Arch. f. Physiol.*, Leipzig, 1890, S. 509), the character of the food in dogs modifies the effects of thyroidectomy. Dogs fed with milk bear the operation better than those fed on flesh, nor do they exhibit, as the latter often do, convulsions after a meal.

[7] *Compt. rend. Soc. de biol.*, Paris, 1891, pp. 841, 843. See also Edmunds, "Proc. Phys. Soc.," *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. Hofmeister (*Beitr. z. klin. Chir.*, Tübingen, 1894, Bd. ii. S. 441), and Leonhardt (*Virchow's Archiv*, 1897, Bd. cxlix. S. 341) have also got positive results in rabbits; and G. R. Murray has succeeded in producing symptoms of myxœdema in a rabbit from which he had some time previously removed the thyroids (*Brit. Med. Journ.*, London, 1896, vol. i. p. 204).

[8] Gley, *Compt. rend. Soc. de biol.*, Paris, 1897, p. 181; Vassale and Generale, *Arch. ital. de biol.*, Turin, 1895 and 1896, tome xxv. p. 459; and tome xxvi. p. 61; Edmunds, "Proc. Phys. Soc.," *Journ. Physiol.*, Cambridge and London, 1896, vol. xx.; also *Trans. Path. Soc. London*, 1895, 1896, and *Journ. Path. and Bacteriol.*, Edin. and London, 1896, vol. iii. p. 488. Blumenreich and Jacoby (*Berl. klin. Wchnschr.*, 1896, S. 327) state, on the other hand, that the inclusion or exclusion of parathyroids is immaterial to the result of thyroidectomy (but cf. Gley, *Arch. f. d. ges. Physiol.*, Bonn, Bd. lxvi. S. 308).

the tissues are not consequent on alterations in the nervous system.[1] The most characteristic nervous symptoms are those which have been already mentioned—muscular tremors, passing gradually into clonic spasms, and finally into convulsive attacks (tetany); there is also apathy and unsteadiness of gait, and, with the advance of time, the gradual supervention of a cretinic condition, together with a lowering of the body temperature and a diminution of cutaneous sensibility. The tremors also gradually cease. Monkeys die in from five to seven weeks after the operation. The tremors are of central origin; they disappear on section of the motor nerve (Schiff), but not on removal of the cortical brain area concerned with the movements of the part (Horsley). They also disappear when a voluntary effort is made, and on reflex irritation. In monkeys there is extensor paralysis of the upper limb, and there may occur attacks of functional hemiplegia.[2] The attacks are diminished by administration of potassium bromide.[3] The number of red corpuscles per c.mm. becomes markedly diminished, while the white corpuscles tend to increase in number. The salivary glands become swollen and enlarged, and contain an excess of mucin. Changes in the composition of the blood have also been observed,[4] and in the proportions of the blood gases,[5] and degenerative changes have been described in the kidneys.[6] It has been shown that the excitability of the cortex of the brain, and even of the lower centres, is increased in animals which have suffered from thyroidectomy,[7] but with the supervention of the cretinous condition it is diminished.[8] The metabolic changes which occur are most obvious in the connective tissues, and

Fig. 84.—Monkey deprived of thyroid.—Horsley.

[1] Whitwell (*Brit. Med. Journ.*, London, 1892, vol. i. p. 430) found what he regards as pathological changes in the nerve-cells of the Rolandic area in a case of myxœdema. Marked changes in the nervous elements have also been described as a feature of the condition of cachexia thyreopriva; Kopp, *Virchow's Archiv*, 1892, Bd. cxxviii. S. 290; Langhans, *ibid.*, S. 318; Capobianco, *Internat. Monthly Journ. of Anat. and Physiol.*, 1894, Bd. xi. S. 471 (abstract in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1893, Bd. vii. S. 112); Lorrain Smith and Pembrey, "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, 1894, vol. xv.; Quervain, *Virchow's Archiv*, 1893, Bd. cxxxiii. S. 481. Vassale and Donaggio found degeneration in the pyramidal tract (!) of dogs, after extirpation of the parathyroids (*Arch. ital. de biol.*, Turin, 1896, tome xxvii. p. 129).

[2] Horsley, *loc. cit.*

[3] Gley, *Compt. rend. Soc. de biol.*, Paris, 1892, p. 300.

[4] Halliburton, *loc. cit.*; Ducceschi, *Centralbl. f. Physiol*, Leipzig u. Wien, 1895, p. 359, and 1896, p. 217; also *Arch. ital. de biol.*, Turin, tome xxvi. p. 209.

[5] Albertoni and Tizzoni, *loc. cit.* (see also p. 943).

[6] Rosenblatt, *Arch. d. sc. biol.*, St. Pétersbourg, 1894, p. 53.

[7] Autokratoff (abstract in *Brain*, London, 1890, vol. xxiii. p. 424).

[8] Horsley, *Brit. Med. Journ.*, London, 1892, vol. i. p. 267.

especially in the integument. These tissues become swollen and contain a superabundance of mucin;[1] the integument especially swells and the eyelids become puffy, but at the same time the surface becomes dry, and there is a tendency to the shedding of hairs and of the superficial epithelium. This hyperplastic change is followed, if the animal remains alive for a sufficient time, by atrophic changes. The nervous affection which primarily results is usually accompanied by slight fever. Later on this passes off, and the temperature becomes reduced even to some degrees below normal.[2]

As Schiff originally showed, these effects of thyroidectomy can be temporarily prevented by a graft of thyroid; they may also be caused to disappear either by injection of thyroid juice into a vein or under the skin,[3] or even by taking thyroid juice or raw thyroid by the mouth. The effects of grafts are to all intents and purposes permanent, and it has been found, as in the case of the pancreas, that removal of the graft which has maintained the health of the animal after extirpation of its own thyroid, is speedily followed—as with primary removal of the organ—by the usual symptoms of thyroidectomy. It appears, however, to be somewhat difficult to ensure the graft taking.[4]

Theories of action of thyroid extirpation.—Various theories have been advanced to account for the effects of removal of the gland. H. Munk[5] held that the effects of removal are due, not to interference with the functions of the gland, but to interference with adjoining nervous structures in the neck. But this, as with the similar theory propounded to account for the effects of extirpation of the pancreas, is absolutely negatived if the results of thyroid grafting are to be accepted. Besides this theory, two others, out of the many which have been put forward, deserve consideration.[6] Of these the one may be called the theory of "autotoxication" and the other that of "internal secretion." The autotoxication theory assumes that there are one or more toxic substances constantly tending to accumulate in the blood, and which it is the purpose of the thyroid gland to remove and

[1] F. Semon (*Brit. Med. Journ.*, London, 1883, vol. ii. p. 1073) has enunciated a theory which deserves consideration here, to the effect that removal of the thyroid produces an interference with the full chemical development of the constituents of the connective tissues, so that these tend to take on an embryonic character; and it is well known that excess of mucin is characteristic of embryonic connective tissue.

[2] See on this subject, Horsley, *Brit. Med. Journ.*, London, 1892; Ughetti, *Riforma med.*, Roma, 1890, vol. vi. p. 228.

[3] Vassale, *Riv. sper. di freniat.*, Reggio-Emilia, 1890, tome xvi. p. 439 (abstract in *Centralbl. f. d. med. Wissensch.*, Berlin, 1891, S. 14); and *Arch. ital. de biol.*, Turin, 1892, tome xvii. p. 173; Gley, *Compt. rend. Soc. de biol.*, Paris, 1891, p. 251; G. R. Murray, *Brit. Med. Journ.*, London, 1891, vol. ii. p. 796; 1892, vol. ii. p. 449; 1893, vol. ii. p. 677; Schwarz, *Sperimentale*, Firenze, 1892, vol. xlvi.; *Arch. ital. de biol.*, Turin, tome xvii. p. 330; Chopinet, *Compt. rend. Soc. de biol.*, Paris, 1892, p. 602; Brown-Séquard, *Arch. de physiol. norm. et path.*, Paris, 1892.

[4] v. Eiselsberg, *Wien. klin. Wchnschr.*, 1892, S. 81. For a successful case of thyroid grafting in the human subject, see Macpherson, *Edin. Med. Journ.*, May 1892.

[5] *Sitzungsb. d. k. Akad. d. Wissensch. zu Berlin*, 1887, S. 823, and 1888, p. 1059. See on the subject of Munk's experiments, and also on thyroid grafting, Halstead, *Johns Hopkins Hosp. Rep.*, Baltimore, 1896, p. 373. The bulk of this paper deals with the hypertrophy of the remaining portion which follows the removal of a part only of the thyroid. In a recent paper (*Virchow's Archiv*, 1897, Bd. cl. S. 271) Munk endeavours to maintain his position. He denies that either cachexia or myxœdema necessarily follows thyroidectomy, but in this he is at variance with nearly all other experimenters and with the result of clinical experience.

[6] For older theories regarding the functions of the thyroid, see Horsley, *Brit. Med. Journ.*, London, 1892, vol. i. p. 267 *et seq.*

render innocuous.[1] According to this, the function of the thyroid would be primarily excretory. This view is supposed to be supported by the observation, that the urine of animals becomes, after removal of the thyroid, more toxic than that of normal animals,[2] and that the blood is toxic for other animals, and especially for those which have already had the thyroid removed, although this operation may have been performed only a short time previously, and before the symptoms of thyroidectomy have had time to develop. It is not stated what the probable nature of this substance is, or by what tissues it may be formed.

Effect of thyroid juice.—The "internal secretion" theory would explain the phenomena of extirpation as due to the absence of a secretion which is formed within the thyroid or parathyroids, and passes from them into the blood; a secretion which is necessary for certain of the metabolic processes of the animal body, and especially for those connected with the nutrition of the central nervous system and of the connective tissues. That this view of the function of the thyroid, which was the one given originally by Schiff, is in the main the true one, is shown by the fact that beneficial and not toxic effects follow the exhibition of thyroid juice, both in cases of thyroidectomy in animals and in myxœdema and other affections in man. Moreover, extracts of thyroid gland produce distinct physiological effects in the normal subject.[3] If a decoction of the gland be injected into a vein, the blood pressure markedly falls (Fig. 85), although the beats of the heart remain at about the same rate and of the same strength as before.[4] This lowering of the blood pressure is not, however, peculiar to the thyroid, but occurs with extracts of some other secreting glands. But it has been shown by G. Oliver,[5] that the exhibition of thyroid juice or other preparations of thyroid seems to possess a specific tendency to increase the calibre of the radial artery in the human subject. It would seem, therefore, that the juice of the thyroid, and extracts which are obtained from the gland, have a distinct action upon the vascular system. It has further been noticed that feeding with thyroid tends to cause increased metabolism in the body, accompanied by diuresis and diminution of fat, so that it has been proposed as a cure for obesity.[6] Thyroidectomy alters the conditions of the gaseous exchange,[7] and this in all probability by an indirect effect through the vasomotor system. Lorrain Smith[8] found that in animals which have been deprived of the thyroid body, the reaction to changes of temperature is abnormally rapid. When normal animals are exposed to a cold atmosphere, the production of carbon dioxide becomes increased, consistently with the increased oxidation which is necessary to cause

[1] Horsley, *Proc. Roy. Soc. London*, 1886, vol. xl. p. 6; *Brit. Med. Journ.*, London, 1892; Blumenreich and Jacoby, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. xiv. S. 1.

[2] Laulanié, *Compt. rend. Soc. de biol.*, Paris, 1891, p. 307; see also Gley, *ibid.*, 1894, p. 192; and Masoin, *ibid.*, p. 105.

[3] Gley, *Arch. de physiol. norm. et path.*, Paris, 1894, p. 484.

[4] Oliver and Schäfer, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 277.

[5] Croonian Lectures, *Lancet*, London, 13th June 1896.

[6] Leichtenstern, *Deutsche med. Wchnschr.*, Leipzig, 1894, No. 50. See on the physiological action of thyroid extract, Ewald, *loc. cit.*; Donatti, *Virchow's Archiv*, 1896, Suppl. Bd. cxliv. S. 253; Berkeley, *Johns Hopkins Hosp. Bull.*, Baltimore, July 1897. Berkeley examined the nerve centres of animals which had died from prolonged administration of thyroid extract, but could find no evidence of any changes in the nerve cells.

[7] Michaelsen, *Arch. f. d. ges. Physiol.*, Bonn, 1889, Bd. xlv. S. 622.

[8] *Journ. Physiol.*, Cambridge and London, 1894, vol. xvi. p. 378.

an increased production of heat. This increase of carbonic acid does not take place immediately, but only comes on after a certain period of time; the temperature of the body being in the meanwhile maintained normal by those physical changes which occur in the circulation, and which allow the quantity of blood brought to the skin, and the amount of heat thereby lost from the general surface of the body, to be varied. Now, it is precisely these vasomotor changes which appear to be lacking after removal of the thyroid; for the production of carbon dioxide becomes almost immediately increased by exposing thyroidectomised animals to a low temperature. Cardiac palpitations with increased

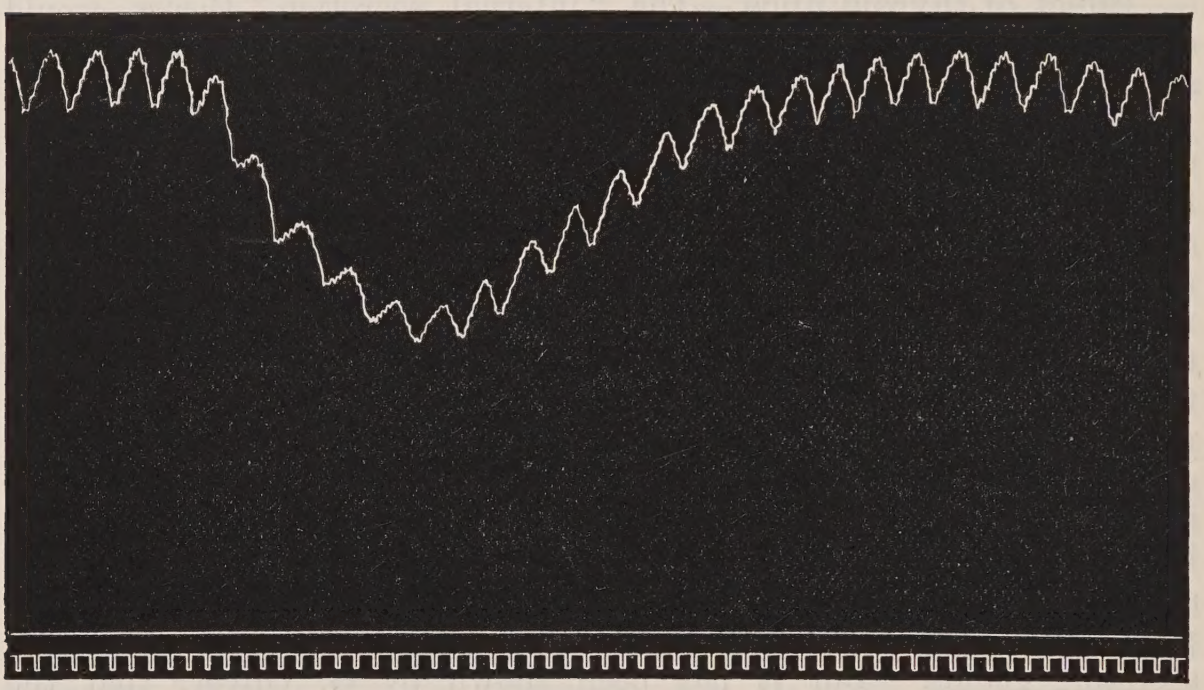


FIG. 85.—Effect in the dog upon the blood pressure of the intravenous injection of decoction of thyroid. Time in seconds. The line above the time tracing is the abscissa of the mercurial manometer.

pulse frequency, often accompanied by a feeling of giddiness, may sometimes be produced by large doses of fresh thyroid; after a time glycosuria and increase of urea appear.[1] Experiments upon the effect of thyroid feeding on metabolism have been made by various observers.[2] Richter[3] found in man no marked effect on nitrogenous metabolism, but Bleibtreu and Wendelstadt and also Roos got a distinct increase of excreted nitrogen during thyroid feeding. Schöndorff obtained an increased excretion of nitrogen during the first eight days (in dog); after that, N-balance was maintained, while the body-fat was greatly diminished in amount. The sodium chloride and phosphoric acid were also somewhat increased. Bettmann[4] states that thyroid feeding tends to produce "alimentary glycosuria" (see p. 881).

[1] Georgiewsky, *Centralbl. f. d. med. Wissensch.*, Berlin, 1895, Bd. xxvii.

[2] Napier, *Lancet*, London, 1893, vol. ii. p. 805; Vermehren, *Deutsche med. Wchnschr.*, Leipzig, 1893, No. 11; Dening, *München. med. Wchnschr.*, 1895, S. 464; Bleibtreu and Wendelstadt, *Deutsche med. Wchnschr.*, Leipzig, 1895, S. 346; Mediger, Diss., Greifswald, 1895 (abstract in *Centralbl. f. Nervenh. u. Psychiat.*, Coblenz u. Leipzig, Bd. xviii. S. 289); Lanz, *Deutsche med. Wchnschr.*, Leipzig, 1895, S. 597; Irsal, Vas, and Gara, *ibid.*, 1896, Bd. xxii. S. 439; Schöndorff, *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiii. S. 423; Gluzinski and Lemberger, *Centralbl. f. innere Med.*, Leipzig, Bd. xviii. S. 90; Roos, *Ztschr. f. physiol. Chem.*, Strassburg, 1895, Bd. xxi. S. 19; and 1896, Bd. xxii. S. 18; Gürber, *Sitzungsb. d. phys.-med. Gesellsch. zu Würzburg*, 1896, S. 101.

[3] *Centralbl. f. innere Med.*, Leipzig, 1896, S. 65.

[4] *Berl. klin. Wchnschr.*, 1897, S. 518.

That the thyroid gland yields an internal secretion which subserves a useful purpose within the body, appears to follow conclusively from these data, and the effects which follow thyroidectomy are probably due to the loss of that secretion. Whether the gland also possesses the function of destroying toxic products of metabolism which would otherwise tend to accumulate in the blood, a function which has been attributed to it by some authors, is a point the evidence regarding which is at present insufficient.

On account of its extreme vascularity and its direct connection with the vessels which supply blood to the head, the thyroid has also been regarded as exercising a regulatory function on the blood supply to the brain,—short-circuiting by vaso-dilatation the cerebral blood flow, or *vice versâ*. This view, which was long previously enunciated by J. Simon,[1] has been of late again brought into prominence by Stahel,[2] whose opinion is supported by that of Waldeyer,[3] both of whom approach the subject from the anatomical standpoint. More recently the matter has been the subject of physiological experimentation by Cyon,[4] who finds that the nerves passing to the thyroid contain powerful vaso-dilatators, and that their stimulation may greatly lower the pressure in the carotid. Cyon further states that they are called into action very easily on excitation of the cut ends of the vagi, of the depressors, or of the cardiac branches of the recurrent laryngeal nerves. After removal of the gland, the excitability of these nerves is diminished, but it is increased by the administration of thyroid preparations.

The mode of connection which unquestionably exists between turgescence of the thyroid and the other nervous and vascular symptoms which characterise Graves's disease (exophthalmic goitre), is still quite obscure. This affection is not, like ordinary goitre and myxœdema, benefited by thyroid feeding; but various observers have obtained considerable benefit by administration of the uncooked thymus of young animals.[5]

The Pituitary Body.

The next organ the internal secretion of which we may shortly consider, is the pituitary body. As is well known, the anterior lobe of the pituitary body is a structure which may in general terms be described as glandular, and although not in all respects resembling the thyroid, there are nevertheless certain points both in connection with its mode of development, and in the structure of the fully formed organ, which might lead to the supposition that there is something functionally common to the two organs.

Effects of removal and disease.—So far as destruction of the pituitary body is concerned, experiments have given interesting results. The organ has been removed successfully in a number of cases in cats by Marinesco,[6] and in dogs by Vassale and Sacchi.[7] In all instances of complete removal death ensued, usually within a fortnight of the

[1] *Phil. Trans.*, London, 1844, p. 295.
[2] *Deutsche med. Wchnschr.*, Leipzig, 1887, S. 227 (quoted by Waldeyer).
[3] *Berl. klin. Wchnschr.*, 1887, S. 233.
[4] *Centralbl. f. Physiol.*, Leipzig u. Wien, 1897, S. 357.
[5] For the literature of this disease, see Ord and H. Mackenzie, in Allbutt's "System of Medicine," 1897, vol. iv. p. 508.
[6] *Compt. rend. Soc. de biol.*, Paris, 1892, p. 509.
[7] *Arch. ital. de biol.*, Turin, 1895, tome xxii. p. 133.

operation. The symptoms observed were—(1) Diminution of the body temperature; (2) anorexia and lassitude; (3) muscular twitchings and tremors, developing later into spasms; (4) dyspnœa. Many of the symptoms show abatement after injection of pituitary extract.[1] Vassale and Sacchi conclude that the pituitary must furnish an internal secretion which is useful in maintaining the nutrition of the nervous and muscular systems. Some of these symptoms, especially the muscular twitchings, are similar to those seen on removal of the thyroid. It has been stated that after thyroidectomy the pituitary body becomes enlarged; and Rogowitsch[2] has supposed that the fact that in rabbits a thyroidectomy sometimes fails to produce the usual results, is due to the pituitary taking on a vicarious action, the pituitary being larger in proportion in the rabbit than in most animals.[3]

Similar statements have been made with regard to its enlargement in some cases of myxœdema, in which the pituitary has been examined. But, on the other hand, Schönemann,[4] who examined the pituitary in a large number of cases of goitre, got no distinct evidence of its enlargement in that disease, nor of any constant change in it, although, in common with other structures, it frequently showed pathological alterations. And whereas enlargement and degeneration of the thyroid is accompanied by cretinism and myxœdema, there appears to be a connection between enlargement and degeneration of the pituitary body and an entirely different disease, to which the name "acromegaly" has been given by Marie,[5] the most obvious symptoms of which are hypertrophy of the bones of the extremities and of the face, with some hypertrophy of the skin and mucous membranes, but without mucinoid degeneration.[6]

Effects of extracts.—The theory that the thyroid and pituitary may act vicariously, appears to be negatived by the physiological effects which are produced by extracts of the last-named gland, and which differ altogether from those furnished by the thyroid.[7] These differences are exemplified in Figs. 85 and 86, which show that, whereas decoction of thyroid produces no obvious effect upon the contractions of the heart, decoction of the pituitary body causes great augmentation in the force of the heart's beat, without, however, any accompanying acceleration of the rate. Further, the effect upon the arteries is precisely the reverse of that which is obtained by extract of thyroid, for, in place of falling, the blood pressure rapidly rises. That this rise is not due simply to augmentation of the heart's beats, but that it

[1] Brown-Séquard, *Compt. rend. Soc. de biol.*, Paris, 1893, p. 527.

[2] *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1889, Bd. iv. S. 453.

[3] See also, on the subject of the possible connection between thyroid and pituitary, H. Stieda, *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1890, Bd. vii. S. 537; Pisenti and Viola, *Centralbl. f. d. med. Wissensch.*, Berlin, 1890, S. 25 and 26; Hofmeister, *loc. cit.*, 1894; de Coulon, *Virchow's Archiv*, 1896, Bd. cxlvii. S. 53; and Leonhardt, *loc. cit.*, 1897.

[4] *Virchow's Archiv*, 1892, Bd. cxxix. S. 310.

[5] *Brain*, London, 1889, vol. xii. p. 59. See also Massalongo, *Centralbl. f. Nervenh. u. Psychiat.*, Coblenz u. Leipzig, 1895, Bd. xviii. S. 281. A. Schiff (*Wien. klin. Wchnschr.*, 1896, Bd. x. S. 277) obtained a marked increased excretion of phosphoric acid on feeding with pituitary tablets, with only a very slight increase of nitrogen. He regards this experiment as indicating an influence of the extract upon the metabolism of bone.

[6] Enlargement of the pituitary only occurred in three cases of acromegaly out of seven described by Souza-Leite (*Neurol. Centralbl.*, Leipzig, 1890, Bd. ix. S. 447), who states that, on the other hand, persistence of the thymus appears to be a fairly constant accompaniment of that disease. Dreschfeld (*Brit. Med. Journ.*, London, 1894, vol. i. p. 6) looks upon the enlargement of the pituitary body as a symptom rather than the cause of acromegaly.

[7] Oliver and Schäfer, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 277.

is also due to contraction of the arterioles, is sufficiently shown by the fact that if salt solution containing pituitary extract be passed through the blood vessels of a frog, the entire nervous system of which has been

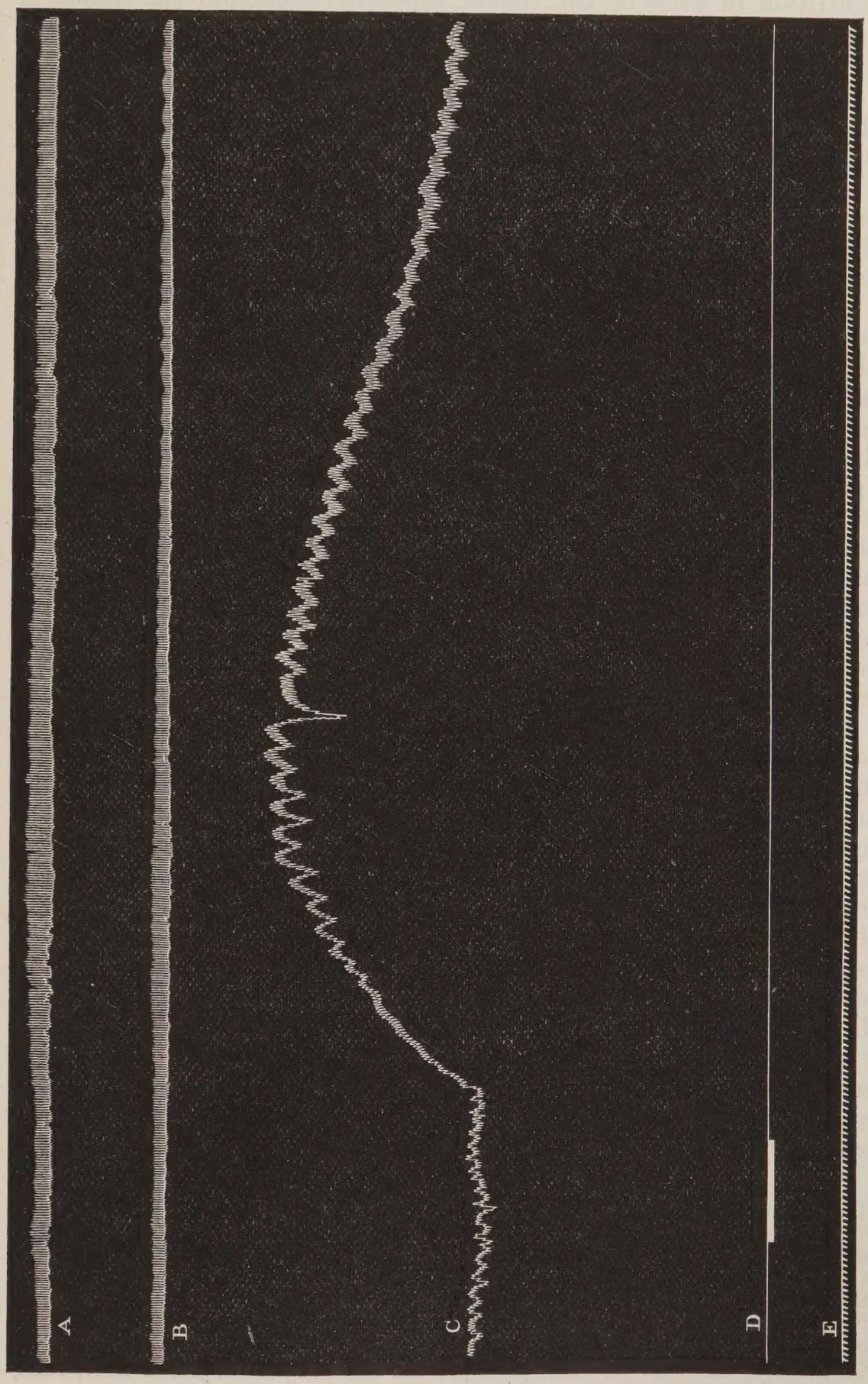


FIG. 86.—Dog, 7 kilos.; morphine, atropine. A, auricle; B, ventricle; C, blood pressure (femoral artery); D, pressure abscissa and signal; E, time in seconds. Effect of intravenous injection of boiled extract, equivalent to 0·07 grm. dry pituitary. (Tracing reduced to one-half.)

destroyed, the vessels markedly contract. This experiment conclusively shows that the effect upon the arteries is a direct one, and in all probability the action upon the heart is also direct.

We may assume, then, that the pituitary body furnishes to the blood an internal secretion, and that this internal secretion tends to increase the contraction of the heart and arteries, and perhaps influences the nutrition of some of the tissues, especially bone and the tissues of the nervous system.[1]

The Suprarenal Bodies.

Effects of disease and ablation.—The immense importance of these glands in nutrition was indicated by Addison,[2] who, in 1855, pointed out that the symptoms of the disease now known by his name, the most prominent of which are extreme asthenia, and the appearance of bronze patches upon the skin and on some of the mucous membranes, are associated with pathological alterations of the suprarenal capsules. This observation was tested experimentally by Brown-Séquard,[3] who found (in 1856) that removal of the suprarenal bodies was rapidly and unfailingly fatal in all animals (usually within twelve hours). Removal of one capsule produces no obvious effect, but when the second is removed, even after a long interval of time, the usual symptoms caused by total ablation at once supervene. The symptoms following the removal are practically those of Addison's disease, although much more acute. There is extreme muscular weakness, and great loss of tone of the vascular system, with loss of appetite, and other signs of general prostration. Death appears to result from paralysis of the respiratory muscles. But the pigmentation which usually accompanies disease of the capsules was not noticed by Brown-Séquard, and he inferred that this absence of pigmentation was probably due to the fact that a fatal result appears so rapidly after the complete removal of the capsules in animals, that time is not afforded for the development of this symptom. This conjecture appears to have been confirmed by an experiment of Nothnagel,[4] who found pigmented patches to appear after crushing the capsules, and also by F. and S. Marino-Zucco,[5] who state that by inoculating the suprarenals of rabbits with pseudo-tubercle bacillus they have succeeded in obtaining, not only the slow development of the ordinary symptoms of suprarenal removal, but also an augmentation in the pigmentation of the skin and hair. Tizzoni also has obtained skin-pigmentation after complete and partial removal of the capsules in rabbits, which lived a certain time after the operation.

It is needless to state that Brown-Séquard's results, following as they did upon Addison's observations, attracted much attention, and numerous investigators set to work to verify them. But many of these[6] failed to confirm the results which were obtained by Brown-Séquard, probably by reason of the removal being incomplete, or of the existence

[1] The thromboses which Mairet and Bosc (*Arch. de physiol. norm. et path.*, Paris, 1896, p. 600) obtained from intravenous injection of glycerin- and water-extracts of pituitary into rabbits, were doubtless caused by nucleo-proteids. Subcutaneous injection produced slight rise of temperature with lassitude and gastric troubles, but as it does not appear that the material used was aseptic, these observations are of little value.

[2] "On the Constitutional and Local Effects of Disease of the Suprarenal Capsules," London, 1855.

[3] *Compt. rend. Acad. d. sc.*, Paris, 1856, pp. 422 and 542 ; *Arch. gén. de méd.*, Paris, 1856 ; *Journ. de la physiol. de l'homme*, Paris, 1858, tome i. p. 160.

[4] *Ztschr. f. klin. Med.*, Berlin, 1879, Bd. i. S. 77.

[5] *Riforma med.*, Roma, 1892, tome i.

[6] Philippeaux, *Compt. rend. Acad. d. sc.*, Paris, 1856 ; Gratiolet, *ibid.*; G. Harley, *Brit. and For. Med.-Chir. Rev.*, London, 1858, vol. xxi. p. 204.

of accessory capsules; and after a few months of controversy the subject gradually dropped, and became for a long time almost forgotten. The interest in this subject has been, however, recently revived, and the experiments of Brown-Séquard have been repeated by various observers (Tizzoni,[1] Abelous and Langlois,[2] and many others). I have myself made several experiments of the same kind on various animals (monkeys, dogs, cats, and guinea-pigs). All these observations have tended to confirm the original statements of Brown-Séquard. They show that animals deprived of their suprarenal capsules die rapidly, usually in the course of one to three days, with the symptoms above noted. The further fact is mentioned by Abelous and Langlois, and this is also confirmatory of a statement of Brown-Séquard,[3] that the blood[4] of animals dying in consequence of the removal of the suprarenal capsules is toxic for other animals which have recently been deprived of their capsules, although it causes no toxic results in normal animals; whereas the transfusion of normal blood into the veins of "decapsuled" animals tends markedly to prolong their survival of the operation.[5]

The symptoms caused by this blood are said by Abelous and Langlois to be those of curari poisoning—paralysis, that is to say, of the intramuscular nerves;[6] and since the most marked phenomena resulting from removal of the capsules is extreme muscular weakness, it has been concluded by them that after removal of these glands a certain toxic product of muscular metabolism accumulates in the blood, and that the function of the glands is to remove or destroy this toxic principle.

This is the "autotoxication" theory of the suprarenal capsules, and is similar to that which has been applied to the thyroid body. Like the other autotoxication theories, it is chiefly founded upon the fact that the blood of animals which are moribund in consequence of the

[1] *Arch. ital. de biol.*, Turin, 1886, tome x. p. 372; *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1889, Bd. vi. S. 1. Tizzoni thought that removal of one capsule only was fatal; this conclusion was shown to be erroneous by Stilling (*Rev. de méd.*, Paris, 1890). Tizzoni found in many of his rabbits alterations in various parts of the central nervous system, apparently brought on by hæmorrhages into the grey matter.

[2] *Compt. rend. Soc. de biol.*, Paris, 1891, p. 835; 1892, p. 388; Langlois, *ibid.*, 1893, p. 444; also in tome iv. of "Travaux du Laboratoire de Ch. Richet," 1897, where will be found a full bibliography (234 papers) and historical account of the subject of the physiology of these organs. Langlois states that it is sufficient to leave $\frac{1}{11}$ of the total weight of the capsules in the dog in order to insure the survival of the animal.

[3] *Journ. de la physiol. de l'homme*, Paris, 1858, tome i.

[4] Also, according to Gourfein (*Compt. rend. Acad. d. sc.*, Paris, 1897, tome cxxv. p. 188), alcoholic extracts of the blood and organs of "decapsuled" animals.

[5] It is stated by Brown-Séquard that injection of extract of suprarenal under the skin of animals the suprarenal capsules of which have been removed, has a partial success in prolonging life (*Compt. rend. Soc. de biol.*, Paris, 1892, tome xliv. p. 410). But it is doubtful if they can be kept alive for any length of time, either by injection in this way or by the taking of suprarenal by the mouth. It appears, however, to be true that some cases of Addison's disease are distinctly benefited by extract of suprarenal capsule, taken by the mouth, but whether any such cases have been cured is doubtful. (For reference to such cases, see Langlois, "Travaux du Laboratoire de Ch. Richet," 1897, tome iv. p. 93 *et seq.*). Abelous (*Compt. rend. Soc. de biol.*, Paris, 1892, Nov. 12) and Gourfein (*Rev. méd. de la Suisse Rom.*, Genève, 1896, p. 113) have succeeded in effecting suprarenal grafts in the frog, which prevented the occurrence of the usual symptoms when the animal's own suprarenals were destroyed; on afterwards removing the graft, the symptoms supervened as usual. Dominicis (*Wien. med. Wchnschr.*, 1897, S. 18), on the other hand, operating on rabbits and dogs, invariably found a fatal result to follow removal of the second suprarenal, after the first one had been successfully grafted.

[6] This statement is, however, denied by Gourfein (*Rev. méd. de la Suisse Rom.*, Genève, 1896, p. 113).

particular extirpation is toxic, especially for other animals which have been submitted to the operation. But it is probable that the blood of an animal dying slowly as the result of any disease, would be to some extent toxic, and the toxic principles would more powerfully affect animals whose resisting power had been lessened by a recent severe operation. However this may be, whether the suprarenal capsules do or do not destroy a toxic principle which is formed elsewhere, and which would otherwise accumulate in the blood, they unquestionably produce a material which has entirely different properties from those stated to be possessed by the blood of animals deprived of their capsules. This material, which is probably the basis of the internal secretion of the glands, has most active physiological properties.

Hypodermic injection of extracts—General effects.—The action upon normal animals of extracts of suprarenal was first investigated by Pellacani and Foà,[1] both alone and in conjunction. They injected subcutaneously extracts of the glands, made with water, and observed the symptoms which resulted. They found that animals (dogs) were killed by subcutaneous injection of extract of calf suprarenal. Their results were criticised by Alexander,[2] who pointed out that there was liability to chemical change in their preparations, and were not confirmed by other observers, but they are, nevertheless, in the main correct.

In conjunction with G. Oliver,[3] I have myself made a number of observations upon the effect of subcutaneous injection of water and glycerin extracts of suprarenal. We found that the animals were usually unaffected by moderate doses, but with larger doses showed quickening and augmentation of the heart-beat, shallow and fast respirations, and fall of temperature. Guinea-pigs, we found, would stand a large subcutaneous dose of suprarenal extract without showing any symptoms at all, or with only a slight acceleration and increase of the force of the pulse. The same appeared to be the case with the cat and with the dog, unless a very large dose were injected, when the symptoms above enumerated became very marked. Rabbits, on the other hand, were more susceptible to the influence of suprarenal extracts. If a large dose were given, the animal succumbed within half an hour. If, on the other hand, the dose was only moderate in quantity, it did not show any symptoms at all for some hours, but then it might suddenly succumb. This primary absence of symptoms was also noted by Foà and Pellacani in dogs. They state that in many of the animals which they experimented upon in this way, there were no symptoms at all apparent upon the day upon which the injection was given, but that the next morning the animal was usually found dead. The cause of death, it may be added, is not by any means clear. Foà and Pellacani have supposed that it may be due to paralysis of the respiratory centre, but the slight effect which intravenous injection of suprarenal extract produces upon this centre does not lend support to this conjecture.

In frogs we found the effect of the water extract or decoction injected into the dorsal lymph sac was to produce a temporary paralysis, which

[1] *Arch. per le sc. med.*, Torino, 1879, 1880, tomes iii., iv., and vii. ; *Arch. ital. de biol.*, Turin, 1883, p. 56.

[2] *Beitr. z. path. anat. u. z. allg. Path.*, Jena, 1892, Bd. xi.

[3] *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. p. 235.

showed itself in very slow and languid movements. This may, however, be due to the veratrine-like effect which the extract produces upon muscular tissue (see below). The subject has been worked at more recently in my laboratory by Swale Vincent,[1] who has performed a large number of experiments upon various animals, and has not only confirmed most of our results, but has added several other facts. Vincent commonly obtained fatal results in guinea-pigs with doses of 6 grms. of fresh gland. In rabbits he found the results to be inconstant. The hind-limbs become paralysed before the fore-limbs in all animals investigated. Doses insufficient to cause a fatal result produce immunity to larger doses which would otherwise be fatal, and this effect may last a few weeks. The action is produced by the medulla of the gland only; extracts of a large number of other organs and tissues were tried, but none produced any effect when injected hypodermically (Vincent).

Intravenous injection.—The intravenous injection of suprarenal extract produces a powerful physiological action upon the muscular system in general, but especially upon the muscular walls of the blood vessels, and the muscular wall of the heart. A certain amount of action is also manifested upon some of the nerve centres in the bulb, especially the cardio-inhibitory centre, and to a less extent upon the respiratory centre.[2]

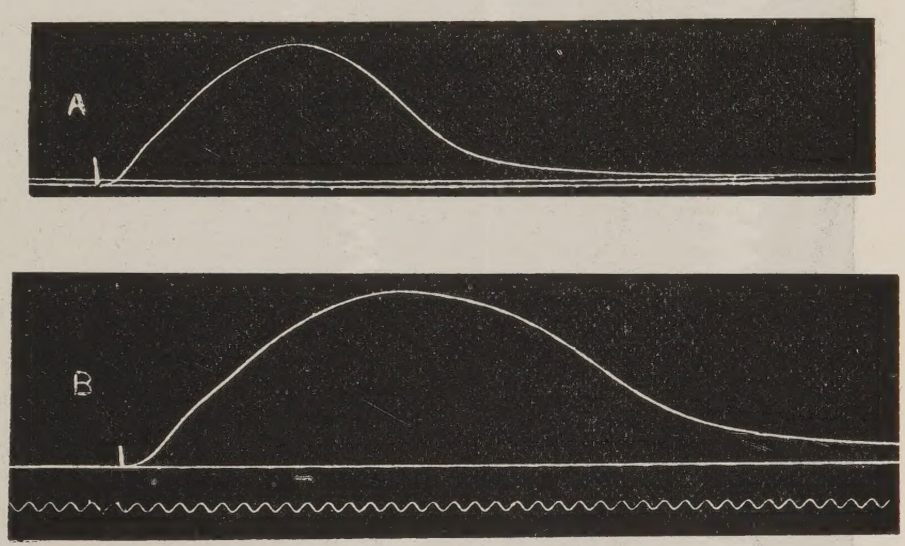


FIG. 87.—Effect of suprarenal extract upon muscle contraction in the frog. A, Normal muscle curve of gastrocnemius; B, Curve taken during suprarenal poisoning, but otherwise under the same conditions as A. Time tracing, 100 per sec.[3]

Action on skeletal muscle.—The effect upon the skeletal muscles is well shown in the frog (Fig. 87), and can also be seen in mammals. The contraction of the muscle in response to a single excitation of its nerve

[1] *Journ. Physiol.*, Cambridge and London, 1897, vol. xxii. p. 111.

[2] Oliver and Schäfer, "Proc. Physiol. Soc.," March 1894 (*Journ. Physiol.*, Cambridge and London, vol. xvi.); "Proc. Physiol. Soc.," March 1895 (*ibid.*, vol. xvii.). These were preliminary communications. The detailed account of the experiments is to be found in the *Journ. Physiol.*, Cambridge and London, vol. xviii. pp. 230–276. The chemical work in connection with our experiments was carried out by Moore; his papers on the subject will be found referred to by Halliburton, on pp. 90–92. Since the first communication to the Physiological Society there have appeared a large number of papers on the subject, for the most part confirming the results there announced. The following are some of these—Szymonowicz, *Anz. d. Akad. d. Wiss. in Krakau*, February 1895; *Arch. f. d. ges. Physiol.*, Bonn, 1896, Bd. lxiv. S. 97; Cybulski, *Gaz. lek.*, Warszawa, and *Anz. d. Akad. d. Wiss. in Krakau*, 1895, reported in *Centralbl. f. Physiol.*, Leipzig u. Wien, 1895, S. 172; Velich, *Wien. med. Bl.*, 1896; Biedl, *Anz. d. k. k. Ges. d. Aerzte, in Wien*, 1896, and *Arch. f. d. ges. Physiol.*, Bonn, 1897, Bd. lxvii.; Gottlieb, *Arch. f. exper. Path. u. Pharmakol.*, Leipzig, 1896, S. 99; Ocaña, *Act. d. l. soc. exp. d. Hist. Nat.*, Madrid, 1897.

[3] Figs. 87, 88, 89, 90, and 91 are taken from the *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii. No. 3.

is as ready as in the normal animal; but it is greatly prolonged, so that the result is comparable to that produced by a small dose of veratria,

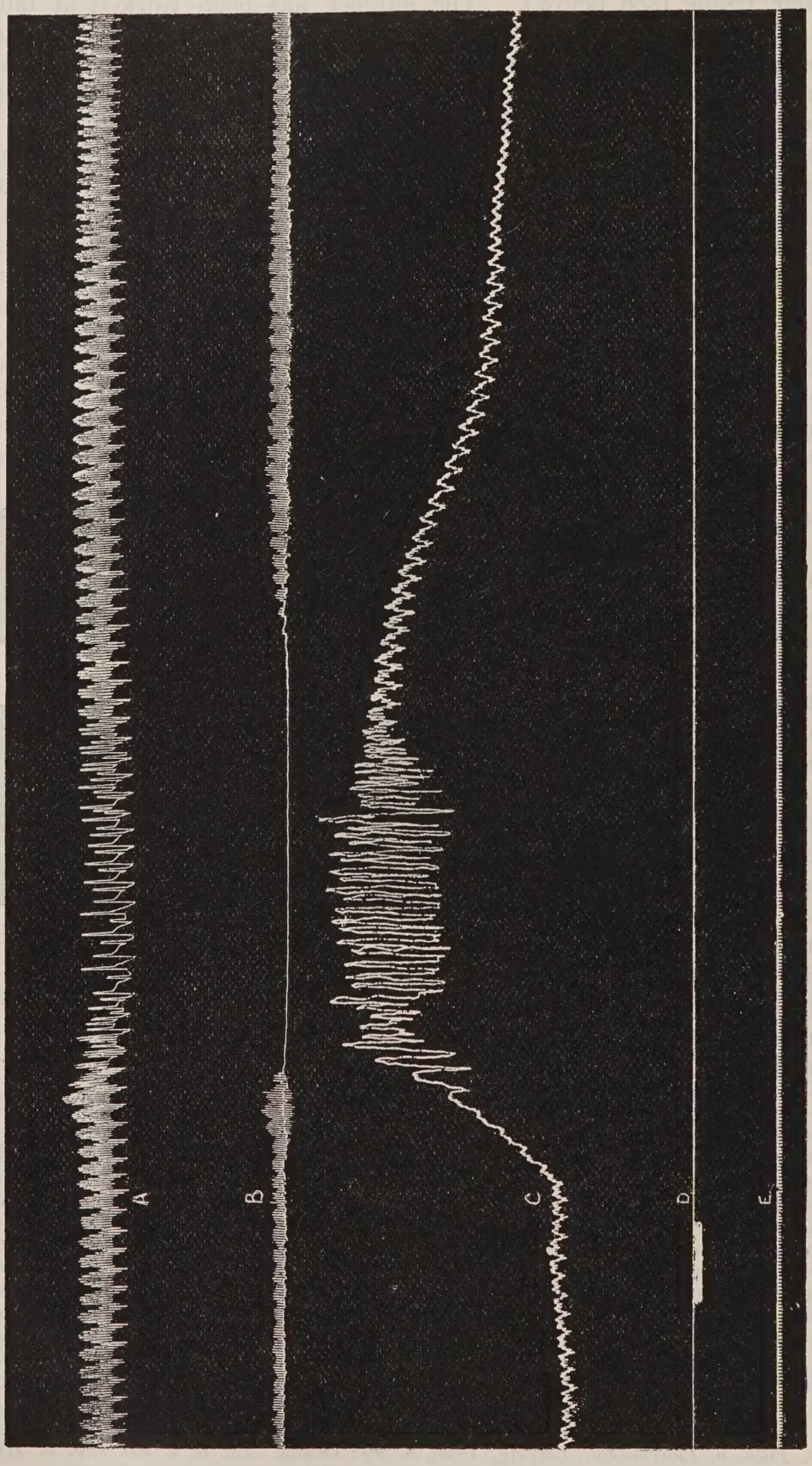


FIG. 88.—Dog of 9 kilos.; morphine, artificial respiration; one vagus only cut. Intravenous injection of 0·2 grms. dog suprarenal. A, ventricle; B, auricle; C, femoral; D, abscissa of blood pressure; E, time, 0·5″. (Tracing reduced to one-half.)

which, as is well known, has the effect of enormously increasing the contraction resulting from a single stimulation of the muscle or its

nerve. It is in no way comparable to a curari effect, for the muscles remain as excitable through their nerves as before. It is therefore an effect entirely different from the so-called autotoxication paralysis which is stated to result after removal of the suprarenal capsules in animals (but see note 6, p. 949), and the material which is extracted by water, therefore, from the suprarenal capsules is certainly not the same material that is said to accumulate in the blood after the removal of those organs.

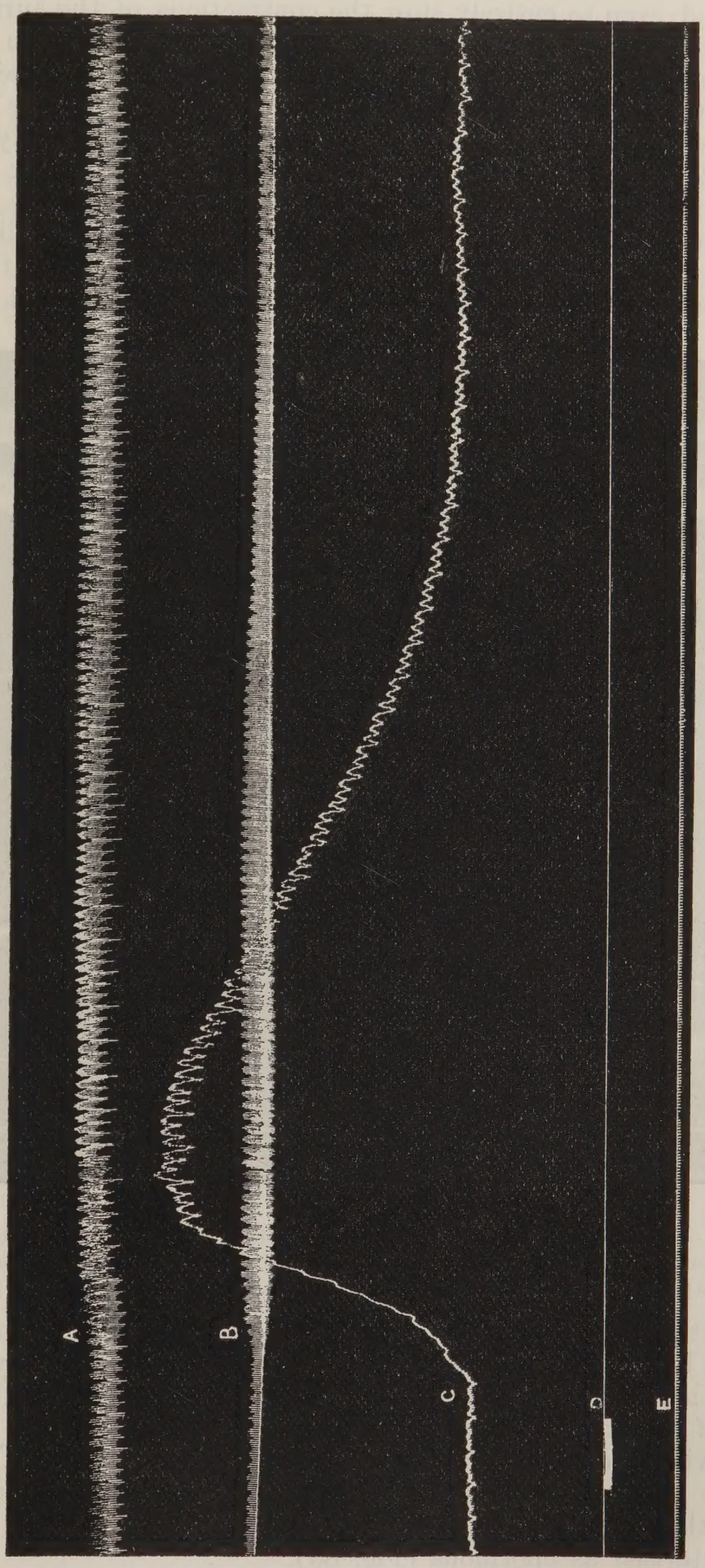


FIG. 89.—Dog, 9 kilos.; morphine, artificial respiration; both vagi cut. A, ventricle; B, auricle; C, blood pressure (carotid artery); D, pressure abscissa and signal; E, time in half-seconds. Effect of intravenous injection of boiled extract, equivalent to 0·2 grms. fresh suprarenal. (Tracing reduced to one-half.)

Action on heart and vessels.—The action upon the circulatory system may be divided into the action upon the heart and the action upon the arterial system. Upon the heart the effect differs, according as the vagi are cut or uncut. When the vagi are uncut

and the heart is therefore still in connection with the cardio-inhibitory centre in the medulla oblongata, the action of suprarenal extract is to slow, and even to entirely stop, the contractions of the auricle. Under these circumstances the ventricle continues beating with an independent slow rhythm (Fig. 88). The result is to cause the pulse to be very slow. On the other hand, when the vagi are cut or their cardiac ends paralysed by atropine, the effect upon the heart is precisely the reverse (Fig. 89). The strength and frequency of the auricular contractions are markedly increased, and those of the ventricle are correspondingly augmented. This naturally has the effect of sending a vastly greater amount of blood into the arteries, which by itself would alone produce a great rise in the arterial pressure. The direct action upon

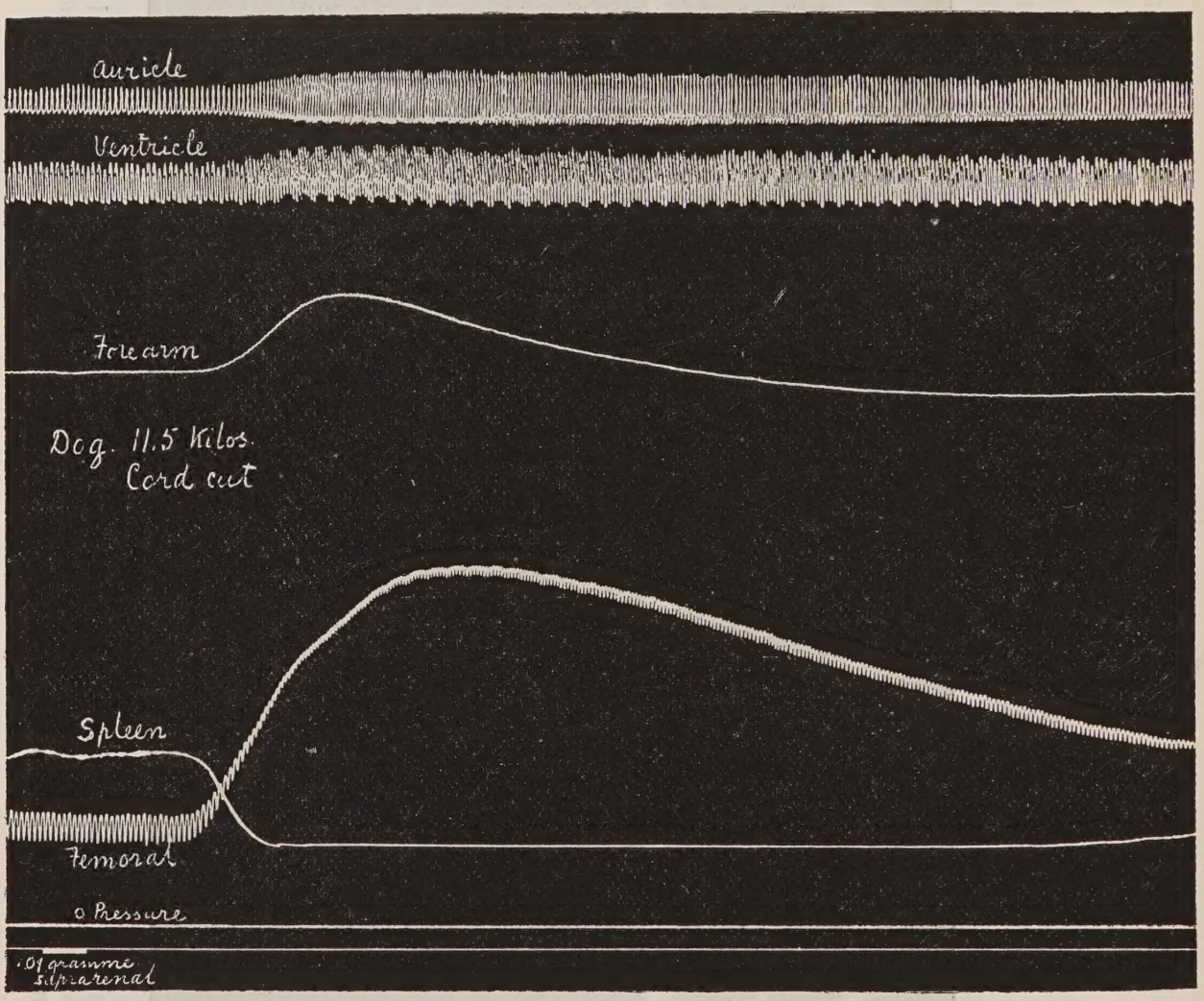


FIG. 90.—Effect of suprarenal extract upon heart, limb, spleen, and blood pressure, after section of cord and vagi. The forearm in this experiment was at first passively expanded, but its contraction is afterwards manifest. (Reduced to one-half.)

the arteries is, however, quite as marked as that upon the heart. If the blood pressure be taken in a dog in the usual way, by connecting a mercurial manometer with the femoral artery, and if a minute dose of suprarenal extract be now injected into a vein, it is found that even with the vagi uncut, and the heart therefore slowed by the action of the extract, the blood pressure rises considerably (Fig. 88). But with the vagi cut or paralysed by atropine the rise can only be characterised as enormous (Fig. 89).

The contraction of the arteries is further exemplified by the fact that if an organ, such as a limb or the kidney or the spleen, be enclosed within

a plethysmograph or oncometer, the instrument indicates a great diminution in volume of the organ, which can only be accounted for by a contraction of its arterioles.[1] This contraction is produced by the direct action of the drug upon the muscular tissue of the smaller arteries, and not indirectly through the vasomotor centre; for it obtains in the mammal equally well with the spinal cord cut or the bulb destroyed (Fig. 90), or even in the case of the arm after the brachial plexus has been severed (Fig. 91). In the frog it is produced also with the brain and spinal cord completely destroyed, when salt solution containing suprarenal extract is allowed to flow through the arteries. Under these circumstances the flow of fluid, which, without the suprarenal extract, may have been comparatively rapid, becomes almost completely stopped, and this can only be due to the direct action of the extractive substance upon the muscular tissue of the smaller arteries.

The enormous rise of blood pressure which is got after the vagi have been cut, is shown in the tracings (Figs. 89 and 90); the pressure may rise to four or five times its original height. Hardly any other agent will produce such an enormous increase of pressure, except direct stimulation of the vasomotor centre. It is not the case, however, that the elevation of blood pressure, and the contraction of the arteries, is due to the stimulation of the vasomotor centre by the drug, as was supposed by Cybulski and Szymonowicz, for, as we have seen, the action is essentially a peripheral one. As shown by Oliver,[2] it will occur if the extract be directly applied to the vessels of the mesentery, either during life or in the "surviving" condition.

The effect of intravenous injection upon the blood pressure passes off in the course of a few minutes. After a dose, no matter whether small or large, has been injected into a vein, and has produced the results which we have recorded, the blood vessels slowly resume their ordinary calibre, the augmentation and increased frequency of the heart's beats become gradually lessened, and the blood pressure recovers its normal condition. Whilst the pressure is raised under the action of suprarenal extract, there is apparently no possibility of inhibiting the arterial contraction; even the strongest stimulation of the depressor nerve, which under ordinary circumstances produces through the vasomotor centre a marked dilatation of the arterioles, is without result during the activity of this extract. The question naturally arises, How is it that the effect so soon disappears? In what manner is the active principle eliminated? It is not eliminated by the kidneys, for the effect passes off just as quickly even although the renal arteries are clamped. It is not eliminated by the suprarenals themselves, for the same fact holds good for the suprarenals. It passes off almost equally quickly if the aorta and vena cava are tied in the upper part of the abdomen, so that there is no circulation of blood whatever in the abdominal organs. It is not oxidised or otherwise destroyed by the blood, for it retains its full potency even after it has been twenty-four hours in contact with that fluid. The most probable explanation of the disappearance of the effect seems to be that the active principle becomes packed away, and eventually rendered innocuous in certain organs. That the muscles take most part in this storage is probable, from the fact that the physiological effects upon the skeletal muscles are manifested for a long time after the effects upon the heart and arteries have disappeared.

[1] In man the effect of taking suprarenal extract by the mouth is to produce a general diminution in calibre of the arteries as measured by the arteriometer (Oliver, "Croonian Lectures," *Lancet*, London, 1896).

[2] "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, March 1897.

Source of the active material.—The physiologically active material is yielded entirely by the medulla of the capsules; no appreciable amount

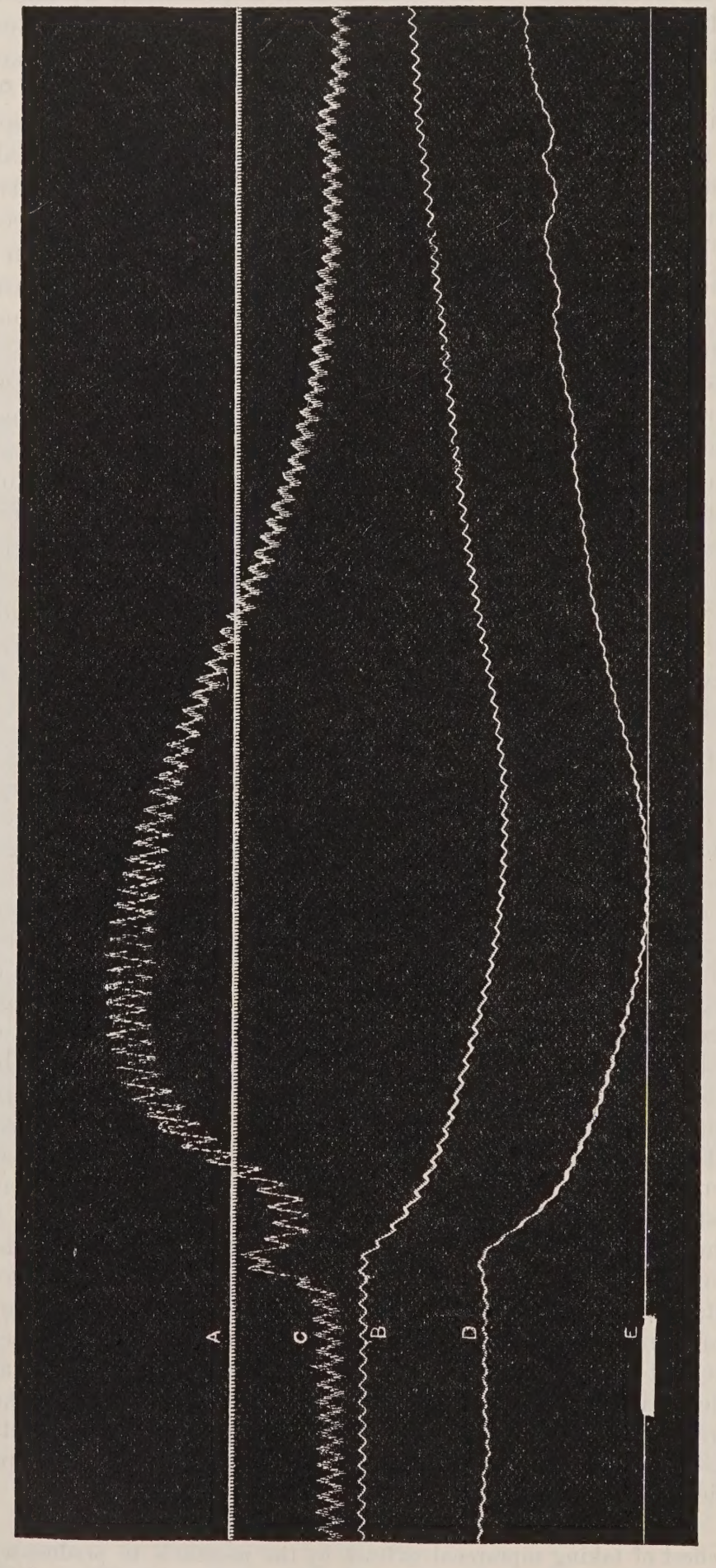


Fig. 91.—Dog of 20 kilos.; morphine, curari; vagi cut; artificial respiration. A, time in 0·5″; B, plethysmographic tracing of right forearm (brachial plexus cut); D, do. of left forearm (plexus uncut); C, blood pressure in femoral; E, abscissa of blood pressure and signal of injection. Effect of intravenous injection of extract of 0·2 grms. calf suprarenal. (Reduced to one-half.)

can be obtained from the cortex. This result, which was arrived at by Cybulski and by ourselves from an investigation of the mammalian

organ, has been confirmed in an interesting manner by the observations of Swale Vincent upon the glands of fishes.[1] Elasmobranchs possess two sets of organs, which appear from their structure to represent the suprarenal capsules of other vertebrates; the one of these, the *inter-renal body* of Balfour, lies between the posterior part of the kidneys in the middle line; the other, the *paired bodies* of Balfour, forms a series lying on either side, segmentally arranged, on the branches of the dorsal aorta. Teleosts possess only one kind of gland representing the suprarenal; this in its structure is similar to the inter-renal of Elasmobranchs. As Vincent has shown, the minute structure of the paired bodies of Elasmobranchs resembles that of the medulla of the suprarenal of other vertebrates, while the inter-renal body is similar to the cortex of the ordinary vertebrate suprarenal.[2] The physiological test shows this in a striking manner, for injection of an extract of the paired bodies of Elasmobranchs produces in a marked degree the phenomena which are characteristic of the medulla of the mammalian suprarenal, while extracts of the inter-renals of Elasmobranchs and of the corresponding organs of Teleosts have no such effect.[3]

Dose.—One of the most interesting and important facts regarding the material which is yielded by the suprarenals, is the minuteness of the dose which is necessary to produce the results. As little as 0·0055 grms. ($5\frac{1}{2}$ mgrms.) of dried suprarenal is sufficient to obtain a maximal effect upon the heart and arteries in a dog weighing 10 kilos. For each kilogramme of body weight, therefore, the necessary quantity to produce a maximal effect is 0·00055 grms., or little more than half a mgrm.[4] The active principle is, however, contained only in the medulla of the gland, not in the cortex, and the medulla in all probability does not form more than one-fourth of the capsule by weight. Of the dried medulla certainly not less than nine-tenths is composed of proteid and other material which is not dialysable, and which otherwise does not conform to the chemical properties which are associated with the active substance of the gland. So that, if we take these facts into consideration, we find that, in order to produce a maximal effect, a dose of not more than fourteen-millionths of a grm. of the active material per kilo. of body-weight is all that is necessary. Now it is certainly true to say that one-fourteenth of this dose will produce some effect, although not perhaps a very large one. We thus arrive at the astounding conclusion, that the active principle of the suprarenal capsules, administered in the proportion of not more than one-millionth part of a grm. per kilo. of body weight, which would be equivalent to $\frac{1}{13000}$ grms. (less than $\frac{1}{800}$ of a grain) for an adult man, is still sufficient to produce distinct physiological results upon the heart and arteries.[5]

[1] *Anat. Anz.*, Jena, 1897, S. 47; "Proc. Physiol. Soc.," *Journ. Physiol.*, Cambridge and London, March 1897, and *Proc. Roy. Soc. London*, 1897, vol. lxi. p. 64; and vol. lxii. p. 176.

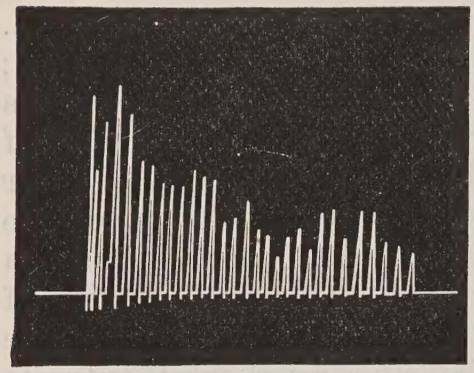
[2] These homologies were long since inferred by Leydig from a study of their structure ("Fische u. Reptilien," Berlin, 1853), and later by Balfour from a study of their development ("Comparative Embryology," 1881, vol. ii. p. 549).

[3] It is, however, difficult to avoid contamination with the paired bodies in extracting the inter-renal. Vincent has also found, in an experiment which is not yet published, that an eel will survive, for some weeks at all events, removal of the glands which appear to be the only representative of the mammalian suprarenals, but contain no medullary tissue.

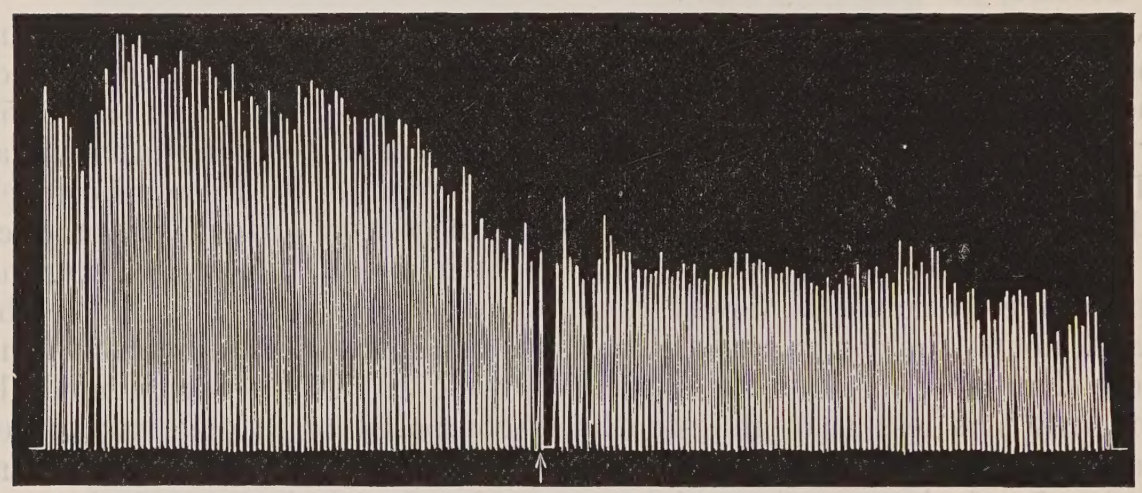
[4] The proportion of suprarenal capsule to body weight is given by Langlois as from $\frac{1}{5600}$ to $\frac{1}{14000}$ in the dog.

[5] The chemical nature of this active principle is still obscure, since, in spite of much work on this subject, it has never been isolated. The history of this has been already dealt with by Halliburton, along with the chemistry of the suprarenals, on pp. 90–92.

Conclusions.—It may be considered probable that the suprarenal capsules are continually secreting into the blood an active material, which, although present in that fluid only in minute quantities, may yet be sufficient to produce very distinct effects upon the metabolic processes of muscular tissue, and especially the muscular tissues of the vascular system. It has, in fact, been stated by Cybulski, and this statement has been confirmed by Langlois and by Biedl,[1] that the blood of the suprarenal vein contains a sufficient amount of the active principle of suprarenal extract to produce a marked rise of blood pressure when intravenously injected. I have, in spite of careful experiments, not been able myself to confirm this statement. Nor is it easy to understand how it can be true, since such blood is constantly flowing



A.



B.

FIG. 92.—*A*. Ergograph tracing of a person suffering from Addison's disease. *B*. Tracing from the same person after six weeks' treatment with suprarenal extract.—Langlois. *A*, natural size; *B*, reduced to one-half.

into the vena cava in larger quantity than these observers injected. But whether we are able to show it experimentally or not, there is very little doubt of the fact that the materials formed pass somehow or other into the blood; and when we compare the results of suprarenal injection with the effects obtained from the removal and from disease of these organs, we can come to no other conclusion than that we have before us a notable instance of internal secretion; and that the effect of such secretion passed into the blood is beneficial to the muscular contraction and tone of the cardiac and vascular walls, and even of the skeletal muscles, appears very evident from the results both of removal of the organs and of injection of their extracts.[2]

[1] *Arch. f. d. ges. Physiol.*, Bonn, 1897, Bd. lxvii.

[2] This conclusion, which is the one arrived at by Oliver and myself, is different from that of Abelous and Langlois, which they formulate thus:—"Les capsules surrénales ont pour fonction de neutraliser ou de détruire des substances toxiques élaborées au cours des échanges chimiques et spécialement au cours du travail des muscles." This statement, which may be taken to set forth the auto-intoxication theory (*supra*, p. 949), was made in 1891, and therefore before the effects of injection of the extract of the medulla were known.

In advanced cases of Addison's disease, with complete degeneration of the medulla of the suprarenals, an extract of these organs is devoid of all physiological activity.[1] Such patients show, as already stated, extreme muscular weakness, and very rapidly become fatigued, and their capability of raising a weight, as estimated by Mosso's ergograph, is extraordinarily small. In one such case, which was treated by exhibition of fresh capsules of the calf, the amelioration of this condition was found by Langlois to be very manifest. The above is well illustrated by the accompanying tracings—Fig. 92, *A*, in a patient with Addison's disease; Fig. 92, *B*, in the same patient after six weeks' treatment with suprarenal capsules of the calf.[2] The tracings are not strictly comparable, for the second one was taken with half the weight, but on the other hand it has been reduced to one-half.

Influence of the Spleen on Metabolism.

The constant occurrence and relatively large size of this organ in vertebrates, the very large supply of blood which it receives, and its intimate anatomical relationships with the digestive organs, would seem to render it probable that it must have important functions to perform in connection with the nutrition of the body.

Effects of removal.—This supposition is not, however, borne out by the result of experiment, for it has been abundantly proved that the spleen can be completely removed in animals and in man without their exhibiting any abnormal symptoms whatever.[3]

Whether the functions of the organ can be taken up under these circumstances by other organs, such as the lymphatic glands, is a point which has not yet been determined. In a dog from which I had removed the spleen several months previously, and which was examined for me with regard to this point by Swale Vincent, there appeared to be a larger number of hæmal lymphatic glands than in normal dogs,[4] but it would require a long series of observations to establish this point conclusively. Certainly such a function as the formation of lymph corpuscles may well be carried on by the abundant lymphoid tissue which is present in other organs of the body; but the spleen has undoubtedly, in addition to this, a certain influence upon the hæmoglobin of some, at least, of the blood corpuscles which are passing through its tissue, for we find hæmoglobin in various stages of transformation into other kinds of pigments within the cells of the organ, and also find a relatively considerable amount of iron in loose organic combination. It has therefore been supposed that the cells of the spleen pulp may produce disintegration of effete red blood corpuscles, and that their pigment may pass to the liver, either as free hæmoglobin or as formed bile pigment. Neither free hæmoglobin[5] nor bile pigment can, however, be detected in the blood of the splenic vein.

On the other hand, the function of producing new red blood corpuscles has been ascribed to the spleen, on the grounds that

[1] Oliver and Schäfer, *loc. cit.*

[2] For other references and observations on the connection between the suprarenals and Addison's disease, see H. D. Rolleston, Goulstonian Lectures, *Brit. Med. Journ.*, London, 1895, vol. i.; and Langlois, "Maladie d'Addison," "Dictionnaire de physiologie de Ch. Richet," Paris, 1895.

[3] There is considerable literature on this subject. It has been collected by Pernet, and is given by him in the *British Medical Journal* for November 26, 1896.

[4] For an account of these organs, see Swale Vincent and Harrison, *Journ. Anat. and Physiol.*, London, 1897, vol. xxi. p. 182.

[5] Schäfer, "Proc. Physiol. Soc.," May 1890, in *Journ. Physiol.*, Cambridge and London, vol. xi.

in some animals, *e.g.* rabbits, after extensive blood-letting, nucleated erythroblasts, such as those which are found in bone-marrow, occur both in the spleen pulp and in the blood of the splenic vein; and that if a spleenless animal is bled, the recovery of the usual percentage of red corpuscles is less rapid than in a normal animal.[1]

Whatever may be the nature of its functions in relation to the blood, it is certain that the organ is in no way essential to the normal nutrition of the body. It is, on the other hand, not at all improbable that the main function of the spleen is to serve a mechanical purpose, answering as a reservoir at certain periods of digestion for the blood which has to pass through the portal system; and the fact that, as was first shown by Roy, the spleen normally exhibits regular rhythmic contractions and dilatations, seems to point to its exercising an influence in assisting the flow of blood through the portal vein, and thus through the liver.[2]

[1] Laudenbach, *Arch. de physiol. norm. et path.*, Paris, 1897, pp. 200, 385, and 893.

[2] The functional connection of the spleen with the vascular system is dealt with in the article on "Circulation" in the next volume. Extracts and decoctions of spleen appear to have no specific effect, either when injected subcutaneously or intravenously. Nor is anything known as to any specific functions possessed by the thymus body or by the carotid and coccygeal glands. Extracts and decoctions of the thymus appear to have no specific effect when injected intravenously (Oliver and Schäfer, *Journ. Physiol.*, Cambridge and London, 1895, vol. xviii.) or subcutaneously (Vincent, *ibid.*, 1897, vol. xxii.). It has been stated that removal of the thymus in frogs is followed by a fatal result (Abelous and Billard, *Arch. de physiol. norm. et path.*, Paris, 1896, p. 898), but the statement requires corroboration.

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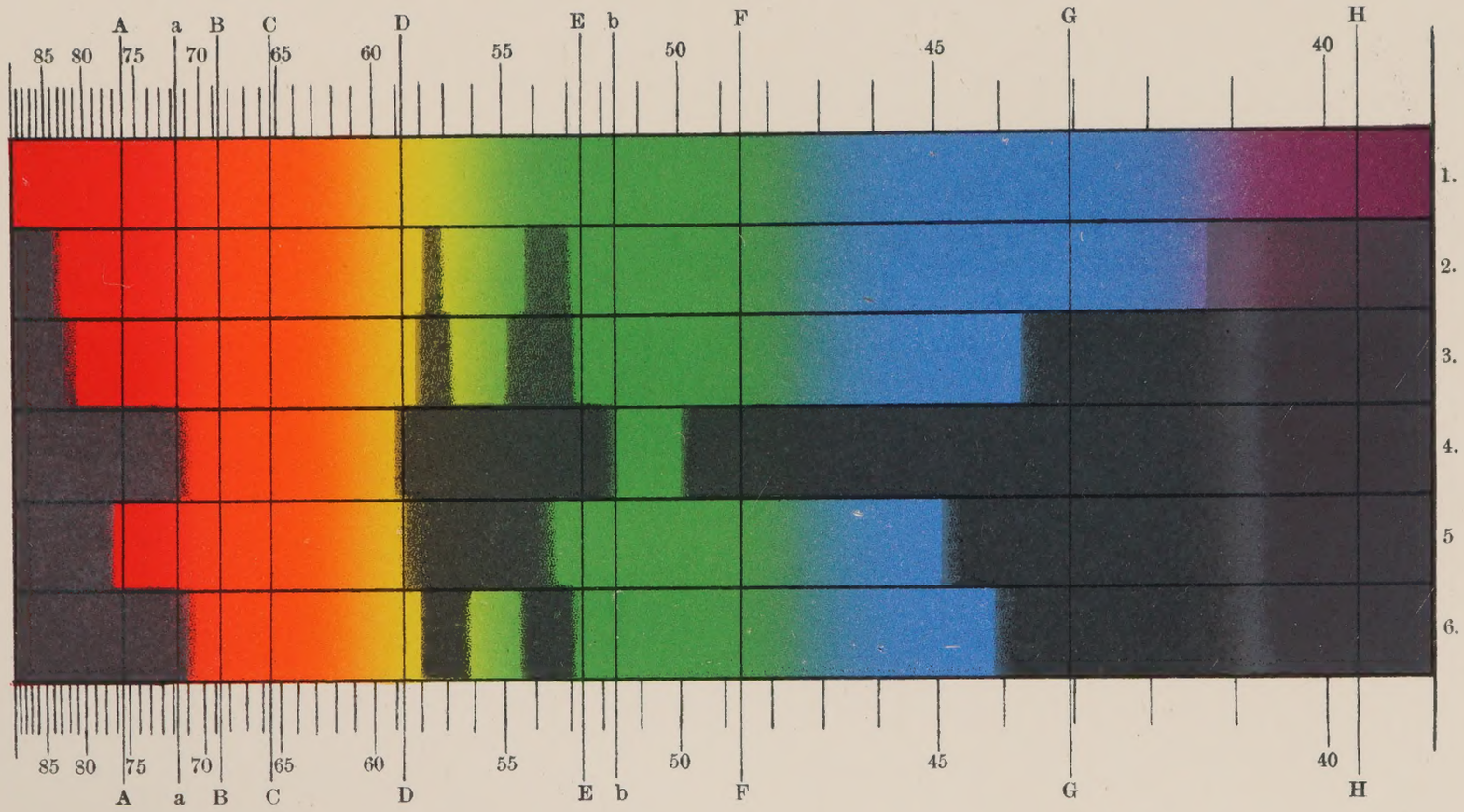
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5 Hæmoglobin (reduced).
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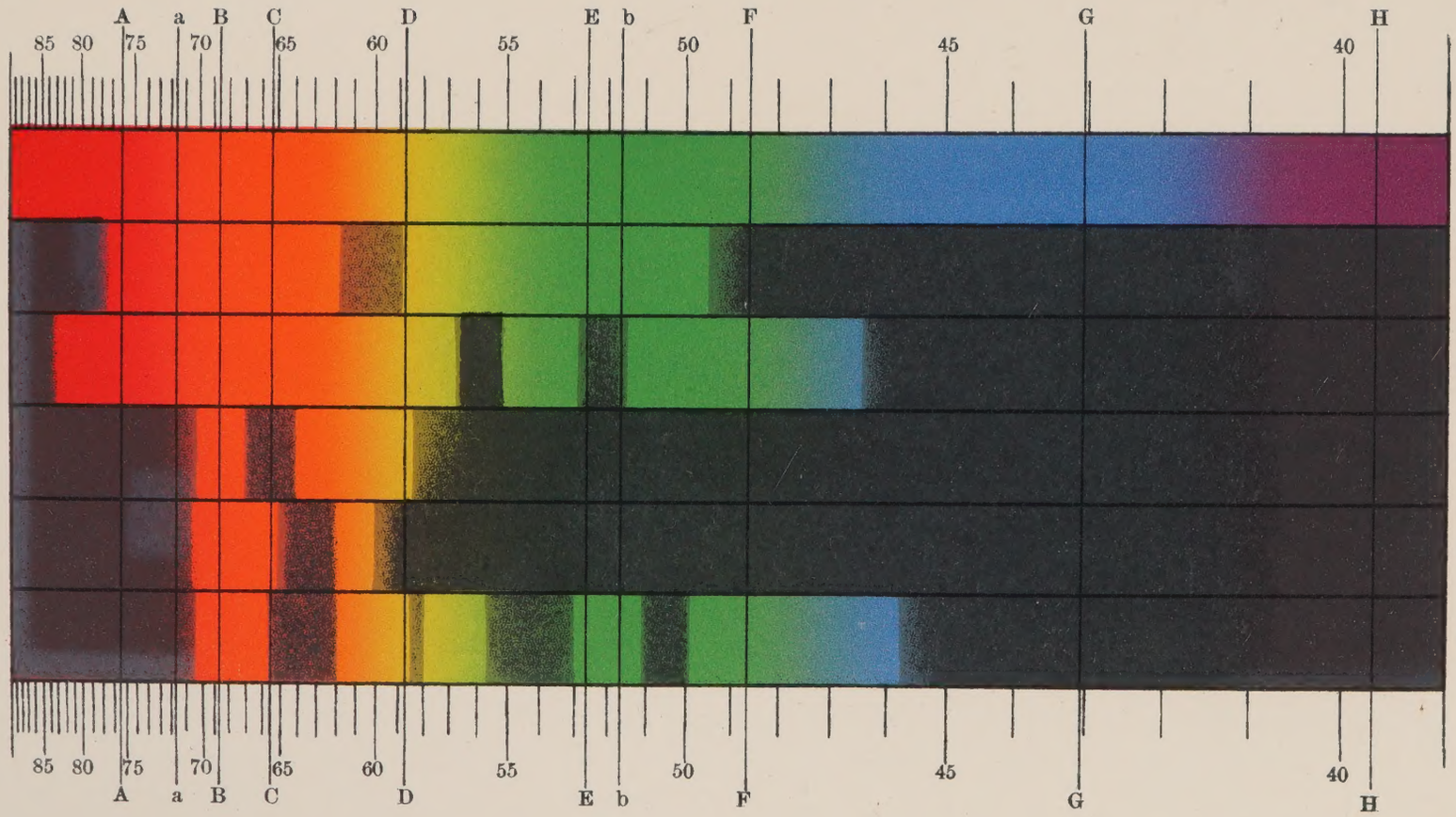

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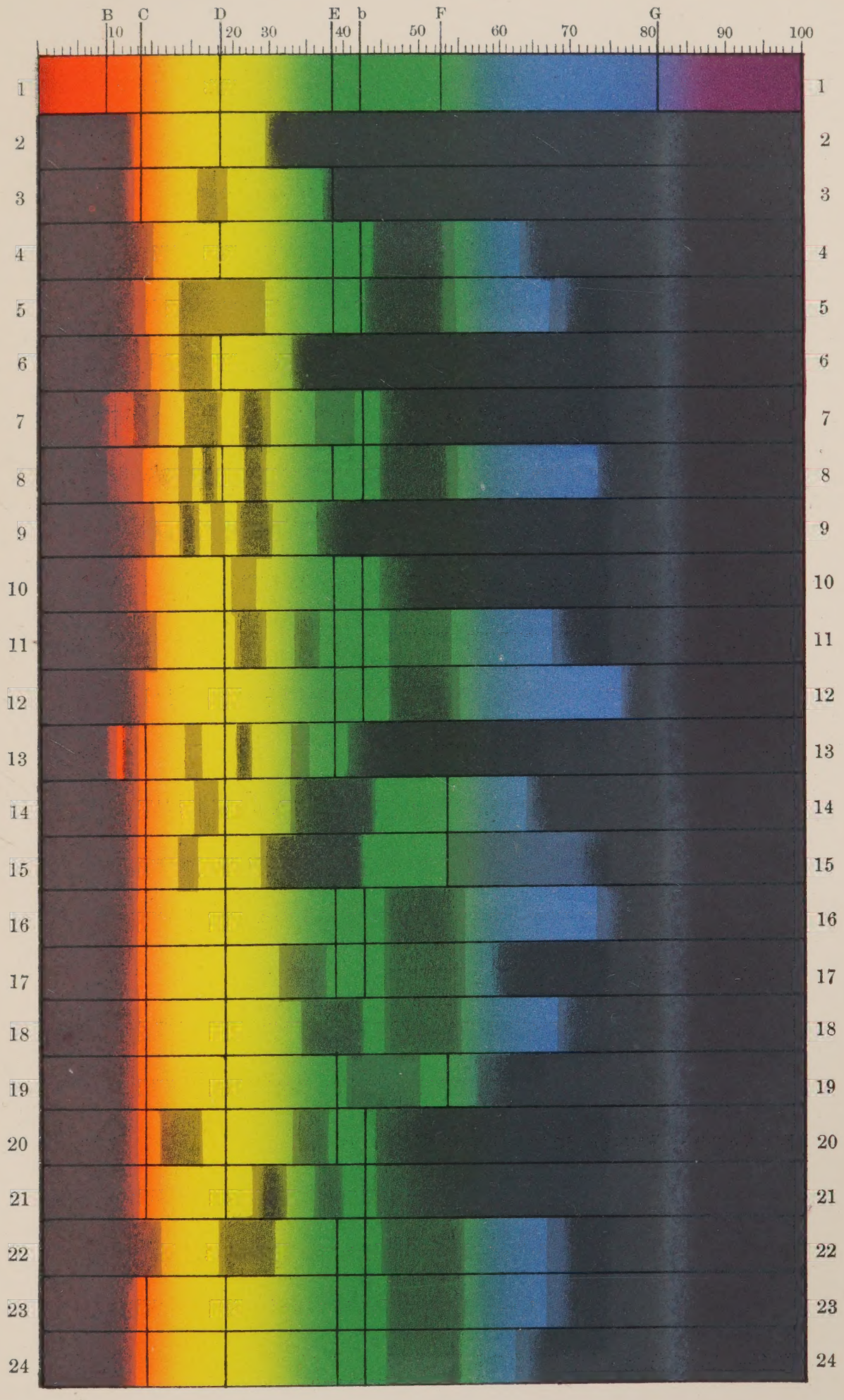
1. Solar spectrum.
2. Hæmatin in alkaline solution.
3. Hæmochromogen.
4. Acid Hæmatin.
5. Methæmoglobin.
6. Acid hæmatin in ethereal solution.

PLATE II.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